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DIAPORTHE PHASEOLORUM VAR. SOJAE ON CROP PLANTS[1]

E. S. Luttrell

(Accepted for publication March 10, 1947)

Pod blight of lima bean, first reported in 1891, is a widespread and occasionally serious disease along the Atlantic Seaboard. In 1917 Harter (4) demonstrated by means of inoculations that *Diaporthe phaseolorum* (C. & E.) Sacc. is the cause of this disease and also established the connection between the pycnidial and perithecial stages of the fungus. *D. phaseolorum* has subsequently been reported on snap bean (14), pepper (16), and tomato (2, 5).

In 1920 Wolf and Lehman (18) reported a similar pod and stem blight of soybean in North Carolina caused by an undetermined species of *Phoma*. Lehman (8) subsequently produced the perithecial stage of this fungus in culture and named it *Diaporthe sojae* Lehman. He reported that successful inoculations had demonstrated its pathogenicity on soybean. Several years later Wolf and Lehman (19) discovered the perithecial stage of the fungus in the field on over-wintered soybean stems. In a taxonomic study of the genus *Diaporthe*, Wehmeyer (17) reduced *D. sojae* to varietal rank, making it *D. phaseolorum* var. *sojae* (Lehman) Wehmeyer.

The pycnidial stage of *Diaporthe phaseolorum* var. *sojae* has been reported frequently on soybean in various parts of the United States and in the Orient. It has been observed also on cowpea (9, 15), peanut (1), snap bean (9), and lima bean (9). Opinions concerning its importance on soybean have been varied. On the one hand, it has been considered merely a saprophyte (11) or a secondary invader of plants killed by other diseases (6, 12); on the other, it has been reported (3) as causing the death of 20 to 35 per cent of the plants in certain fields. The majority of observers, however, have reported only a trace of damage or losses of up to 0.5 per cent of the crop resulting from infection with this fungus.

In Georgia a fungus determined as *Diaporthe phaseolorum* var. *sojae* was found on soybean, snap bean, cowpea, peanut, lupine, lespedeza, *Strophostyles helvola* (L.) Britton, pepper, tomato, okra, onion, and garlic. The fact that what appeared to be the same fungus was found also on lima bean, the host of *D. phaseolorum*, raised the question of the identity of this fungus as well as the question of whether *D. phaseolorum* and *D. phaseolorum* var. *sojae* may be considered distinct varieties, especially since cross inoculations with the two fungi apparently have never been attempted. A study concerned with the identity and importance of this fungus on the various crop plants on which it has been found was made, therefore, during the period

[1] Paper No. 159, Journal Series, Georgia Experiment Station.

I am indebted to Dr. B. B. Higgins, Georgia Experiment Station, Dr. J. L. Weimer, Bureau of Plant Industry, and Dr. S. G. Lehman, North Carolina State College of Agriculture and Engineering, for their suggestions and criticisms of the manuscript and to Dr. L. E. Wehmeyer, University of Michigan, for his advice on taxonomic problems.

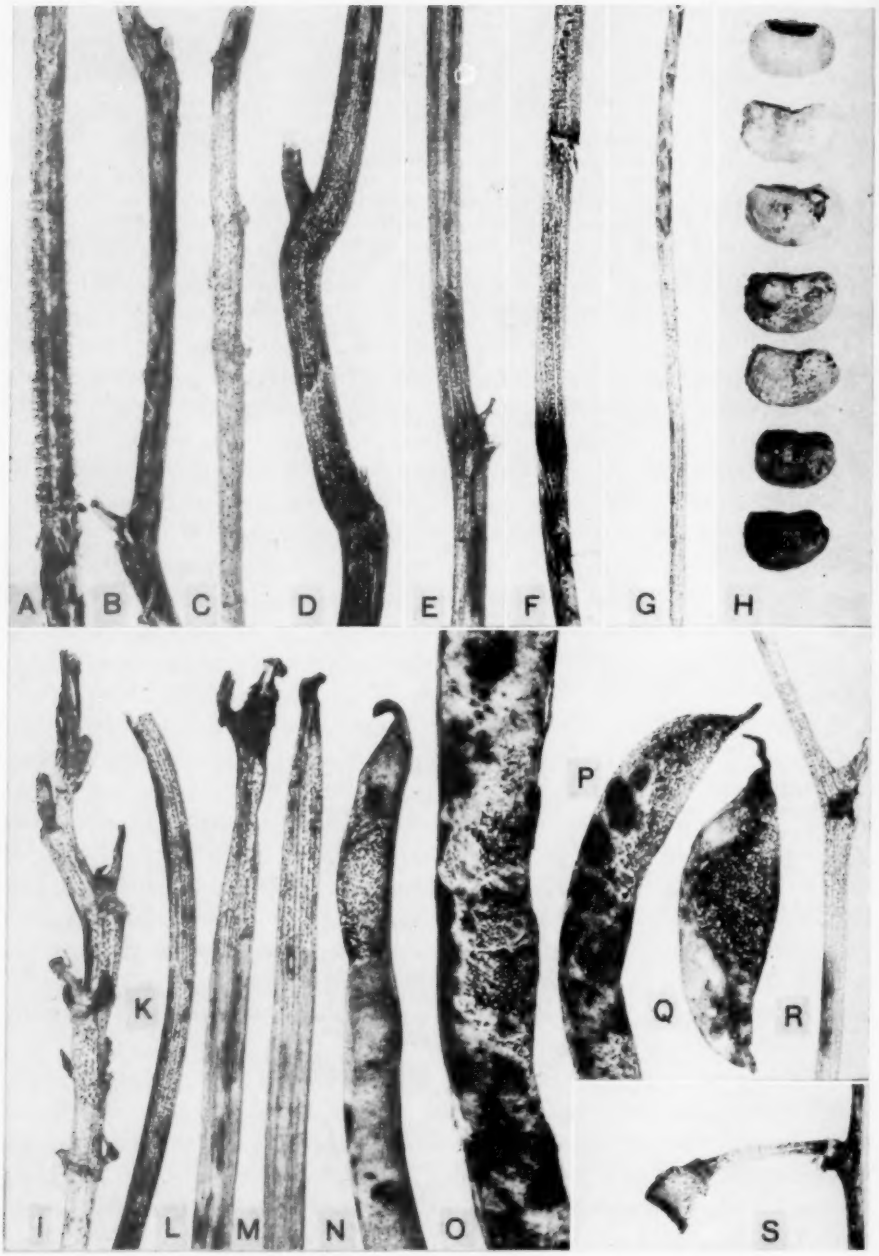

FIG. 1. *Diaporthe phaseolorum* var. *sojae*. A. Perithecia formed on soybean petiole in culture; the perithecial necks developed on the moist lower part of the petiole are long and coiled, while those toward the drier upper part become progressively shorter. ×1. B. Perithecia developed on over-wintered snap-bean stem; the short perithecial necks are barely visible. ×1. C. Pycnidia on stem of snap bean inoculated in greenhouse. ×1. D. Pycnidia on stem of snap bean from the field. ×1. E. Pycnidia on soybean stem. ×1. F. Pycnidia on peanut stem. ×1½. G. Pycnidia on lima-bean stem. ×1½. H. Pycnidia on cowpea seed in various stages of decay. ×1. I. Pycnidia on stem of cowpea inoculated in greenhouse. ×1. K, Pycnidia on stem of cowpea from the field. ×1. L, M. Pycnidia on dead tips of cowpea peduncles. ×1. N. Pycnidia on cowpea pod. ×1. O, P, Q. Pycnidia on snap-bean pods. ×1. R. Pycnidia on snap-bean stem. ×1. S. Pycnidia on snap-bean pedicel. ×1½.

from 1943 to 1946 at the Georgia Experiment Station. This study involved a comparison of the morphology, growth in culture, pathogenicity, and host range of *D. phaseolorum* and *D. phaseolorum* var. *sojae*.

HOSTS AND SYMPTOMS

Snap bean. Although *Diaporthe phaseolorum* var. *sojae* occurs chiefly on the stems and pods, it has been found also on the pedicels, leaves, and hypocotyls of snap bean. It first appears on the pedicels of mature plants. Pedicels, from which the pods have been picked or on which the pods have been damaged by insects, die, turn brown, and become covered with small, black, round to elliptical pycnidia which tend to be arranged in rows along the pedicel (Fig. 1, S). Occasionally pedicels bearing immature pods which are dead and shriveled are similarly infected. Rarely the fungus may spread from the pedicels into the peduncle. It does not spread into the vegetative branches, however, until the pods are mature and dried and the plant becomes senescent. As the plant dies the fungus may spread down the stem and fruit on any part. Pycnidia, arranged usually in longitudinal rows, may cover the entire stem (Fig. 1, C, D, R) or occur in localized patches. The fungus may occur on old mature leaves. Then, irregular, brownish, unbordered dead areas covered with pycnidia extend inward from the margins of infected leaflets. *D. phasolorum* var. *sojae*, associated with *Macrophomina phaseoli* (Maubl.) Ashby and *Fusarium spp.*, has been isolated also from the hypocotyls of snap-bean plants dead apparently as a result of root rot. It has been found once fruiting upon a buff-colored, brown-bordered lesion involving the epicotyl and extending down one side of the hypocotyl of a dwarfed, month-old seedling. Generally, however, infection is found only after the pods have dried and the plants begin to die naturally. Pycnidia then appear on the dead stems of plants which have showed no previous infection. Dried pods are similarly invaded. Pycnidia may be more or les scattered over the surface of the pods or may develop only in patches (Fig. 1, O, P, Q). Often they are densely aggregated to form an almost continuous black crust. They are distributed irregularly instead of being arranged linearly as they are on the stems. Seed in infected pods may appear normal or may be shriveled and nonviable. If the pods are moistened by frequent rains or by contact with the ground, the seed may be covered with a white mycelial weft and almost completely decayed.

The fungus occurred indiscriminately on hybrids of a number of snap-bean varieties at both Tifton and Experiment. At both places it was generally associated with *Macrophomina phaseoli*. It was first observed in a field at the Coastal Plain Experiment Station at Tifton on June 21, 1943, on pedicels of plants that otherwise were healthy and on the stems of plants that had matured and died. In the same field in 1944 it was found again on June 1. Observations were made then on five plots of snap bean of the Tendergreen variety. Two pickings of green beans had already been made at the time, but the majority of the plants were still green and bearing

blossoms as well as pods in various stages of maturity. In a sample of 564 of these plants, 78 per cent were green and still bearing. Of these green plants, 42 per cent showed infection of the pedicels with *Diaporthe phaseolorum* var. *sojae*. Of the 22 per cent that were dead, apparently as a result of root rots, pycnidia were found on 10.5 per cent. It was also isolated from hypocotyl tissue of six of a sample of 20 dead plants on which no fruiting bodies of any fungus were evident. Although pedicel infections were not noticed at Experiment, single infected leaves were found here twice during the 1944 growing season. The fungus was commonly present on dead stems and pods at the time of harvesting for seed at both Tifton and Experiment in 1943. It was much less abundant at the same time in 1944, 1945, and 1946.

Cowpea. *Diaporthe phaseolorum* var. *sojae* has been observed only upon the pods, and, generally to a lesser extent, upon the stems of cowpea. It appears when the first pods become mature and dry. Pycnidia are then found scattered or grouped in patches on the surface of the dried pods (Fig. 1, N). Seed within infected pods may appear normal, or they may be shriveled and killed. Under moist conditions they may be covered with a white mycelial weft. Pods left hanging on the dead plants until late in the season when the pod walls become thin and cracked may contain seed covered with spherical black pycnidia embedded in the mycelial weft over the surface of the seed (Fig. 1, H). From infected pods the fungus spreads into the peduncles as they die. Pycnidia arranged in parallel rows appear on the tips of dying peduncles when the remainder of the plant is green and healthy (Fig. 1, L, M). As the entire plant dies pycnidia develop in patches along the dead stems (Fig. 1, I, K). On cowpea also *D. phaseolorum* var. *sojae* is usually associated with *Macrophomina phaseoli*.

During the fall of 1943 at Experiment only a few scattered infected plants were found. A greater number was found in a large planting of cowpea near Arabi examined in August, 1944, when, although the stems were still green, the majority of the pods were dry and over half of the leaves had been shed. Although stem infections were not common, infection of pods was general. During September and October, 1944, in several plantings of cowpea at Experiment, infection of both stems and pods was general. In one field the infection was greater on cowpea than on soybean and snap bean planted in adjacent rows. Infection was not severe enough to make significant observations on varietal susceptibility. Differences in amount of infection on different varieties seemed to be attributable to the fact that the varieties had matured at different times and, consequently, under different environmental conditions. During September, 1946, pycnidia were found on stems of plants killed by *Sclerotium rolfsii* Sacc. or by *Macrophomina phaseoli* stem canker in a variety planting at Experiment.

Soybean. The fungus is most common on the stems and petioles of soybean, affecting the pods to a lesser extent. Leaf-blade infections are infrequent. The disease first appears on the petioles of lower leaves that are weakened by shading or attacks of other organisms and upon basal

branches that are broken by the weight of pods, wind, or insect damage. Reddish-brown patches of discolored tissue appear on many of these stems, and the stems gradually die. The discoloration appears, however, to be the result of attack by anthracnose. *Colletotrichum glycines* Hori fruits upon the surface of such areas as the stem dies, and pycnidia of *Diaporthe phaseolorum* var. *sojae* then develop upon the intervening areas on the dead stems. *Diaporthe* has not been observed to infect the main stems or upper branches of vigorous plants until after the death of the entire plant following maturity or attack by other organisms such as *Sclerotium rolfsii*. Under favorable conditions dead stems may be covered with pycnidia (Fig. 1, E); while, if conditions are unfavorable, the pycnidia may be limited to small patches, usually formed around the nodes. Pycnidia are often present upon mature dried pods, and the seed in infected pods may be shriveled and dead. Pycnidia occur also upon dried immature pods. Many dried young pods are found, however, whose death appears to be the result of physiological conditions or attack by anthracnose, and it is impossible to be certain that *D. phaseolorum* var. *sojae* is a primary cause of the death of immature pods. On leaves it is sometimes found fruiting upon brownish, unbordered areas of dead tissue extending inward from the tips or margins of the leaflets. *Colletotrichum glycines* is commonly associated with *D. phaseolorum* var. *sojae* on both pods and stems, and no definite lesions which could be ascribed to the latter have been found on soybean.

In the fall of 1943 at Experiment some varieties in a variety planting of soybean were heavily infected, while the majority showed only slight infections. Pycnidia were present on approximately 70 per cent of the dead stems of Boone, the most severely affected variety. Boone was, however, the earliest variety represented in the planting. The pods were mature and dry, and the heavy infection of this variety could be attributed to the fact that it had matured and naturally died earlier than other varieties. During 1944, 1945, and 1946 in the same locality only a few infected pods and stems were found.

Lima bean. *Diaporthe phaseolorum* var. *sojae* has been found only upon dead branches and pods of lima bean (Fig. 1, G). The seed in dried pods may be infected and shriveled when there is no evidence of the fungus on the exterior of the pods. In October, 1943, a few plants of Henderson bush lima bean with infected branches were found at Experiment and on a farm three miles south of Griffin. Pycnidia were present in patches upon the branches, and the fungus was isolated from the tissue of shriveled seed. *Macrophomina phaseoli* also was present on these plants and seemed to be the primary cause of their death. Infection was more abundant on a pole lima bean at Experiment during 1944 and 1945. Pycnidia were present on dead branches of otherwise healthy plants throughout the bearing period. The evidence indicated, however, that *Colletotrichum truncatum* (Schw.) Andrus & Moore was primarily responsible for the death of these branches. Although the dead part of the stem was covered with pycnidia of *D. phaseo-*

lorum var. *sojae*, *C. truncatum* was fruiting at the margin of the lesion toward the base of the branch.

Peanut. Diaporthe phaseolorum var. *sojae* occurs upon the stems and stipules of peanut. Pycnidia develop densely in parallel rows over the entire surface of dead stems, giving them a blackened appearance (Fig. 1, F). They may also be formed on dead stipules on yellowed, moribund stems. In August, 1945, the fungus was found on dead stems of Spanish peanuts at two localities. It did not, however, seem to be responsible for the death of these plants. In a planting near Albany it was fruiting on stems killed by a gopher which had eaten the underground parts of the plants. In a planting near Leesburg it was found on the dead stems of plants killed by lightning. In 1946 it was found by Dr. K. H. Garren on dead stems of Spanish peanuts near Arabi and on dead stems of North Carolina Runner peanuts near Metter. *Sclerotium rolfsii* probably caused the death of these plants.

Lespedeza. Diaporthe phaseolorum var. *sojae* has been found only on dead stems of lespedeza. The pycnidia were produced sparingly on the slender stems but showed a slight tendency toward an arrangement in longitudinal rows. In October and November, 1945, pycnidia were found on a few plants in a field of Korean lespedeza at Experiment. There was no indication that it was responsible for the death of these plants. Although a majority of the plants were dead or dying, only a few stems bearing pycnidia could be found. Death seemed to be the result of natural senescence.

Strophystyles. In 1945 Dr. C. L. Lefebvre pointed out that pycnidia of *Diaphorthe phaseolorum* var. *sojae* were present on the stems in a collection of dead plants of *Strophostyles helvola* made by H. W. Johnson at Americus, Georgia, April 11, 1939. Examination of a duplicate of this collection in the Georgia Experiment Station herbarium showed that pycnidia of this fungus were scattered on the upper parts of stems of what appeared to be seedling plants. Cause of the death of these plants is unknown.

Lupine. In the spring of 1945 and again in 1946 *Diaporthe phaseolorum* var. *sojae* was found on dead stems of blue lupine at Tifton. The lupine had been planted as a winter crop in a pecan orchard. Pycnidia were observed on stems left standing or on the surface of the ground after the crop had been disced under.

Pepper. Diaporthe phaseolorum var. *sojae* has been found on the fruit and the peduncles of pepper. It seems to be capable of acting as a wound parasite, causing a secondary rot of the fruit. The lesions are soft, circular or elliptical, somewhat depressed, and wrinkled. At the advancing margin of the lesion the tissue is blanched and water-soaked. Toward the center it is dry, either blackened or bleached to pale tan, and covered with masses of black pycnidia (Fig. 2, B, D). The pycnidia tend to be arranged in concentric circles, and this arrangement, together with the wrinkling of the host tissue, gives the lesion a zonate appearance. The inner surface of the fruit and the seed are covered with a white mycelial weft. As the rot pro-

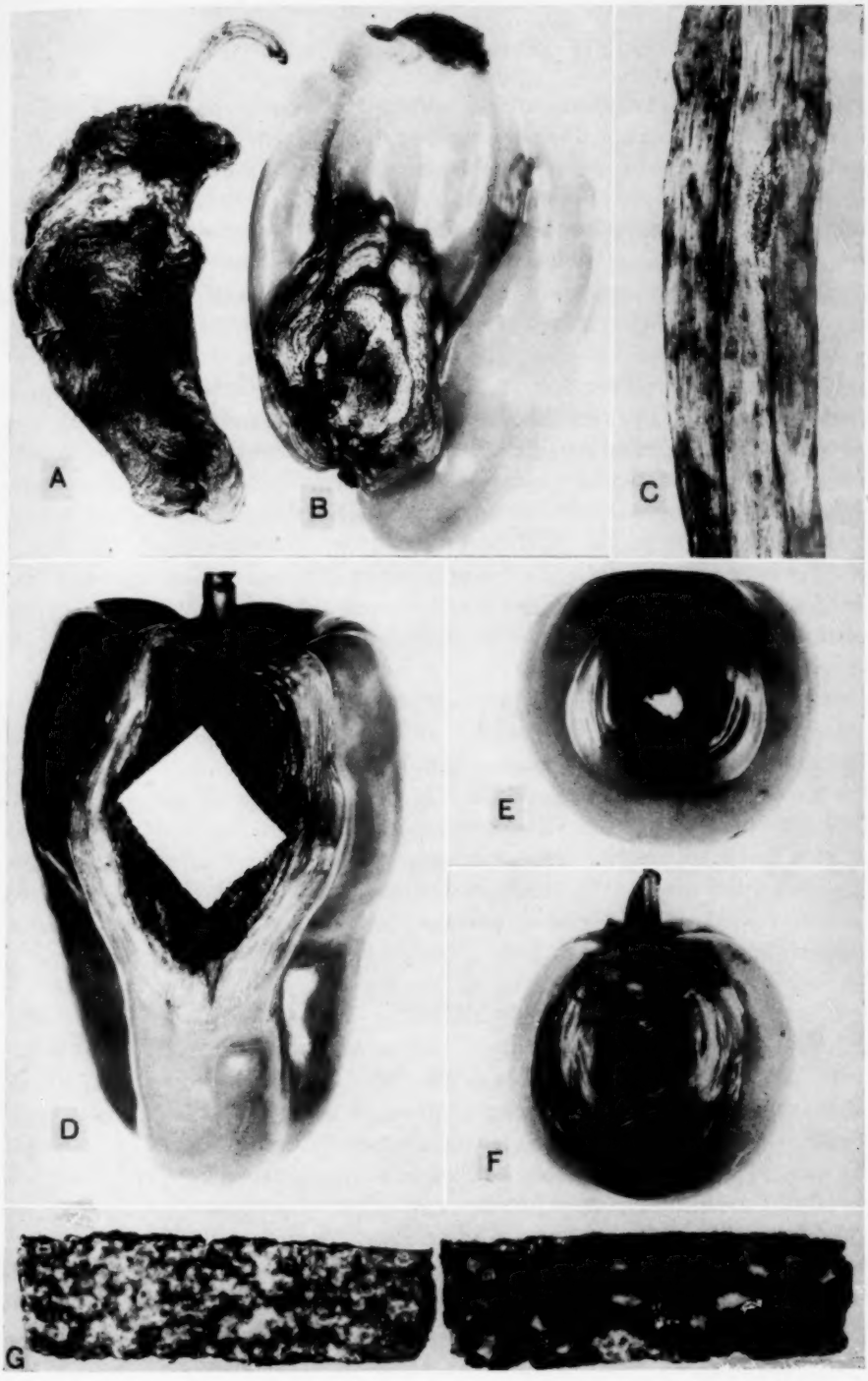

FIG. 2, A–G. *Diaporthe phaseolorum* var. *sojae*. A. Pepper fruit decayed by the fungus after invasion through blossom-end rot spot; pycnidia are on the fruit and on the dead pedunele. ×¾. B. Rot spreading from sunscald spot on pepper fruit; pycnidia have developed in the dead tissue. ×¾. C. Pycnidia on dried okra pod. ×1. D. Lesion produced on pepper fruit in the field by inoculation through wound now covered with adhesive tape. ×¾. E. Lesion on picked tomato fruit in moist chamber produced by inoculation through wound. ×¾. F. Lesion on tomato fruit in the field resulting from natural infection through sunscald spot. ×¾. G. Cultures producing pycnidia of *D. phaseolorum* (left) and *D. phaseolorum* var. *sojae* (right) on cornmeal mush. ×1.

gresses the fruit shrivels to a dry, wrinkled mass covered with a crust of pycnidia (Fig. 2, A). The fungus may then invade and fruit upon the peduncle (Fig. 2, A). It was fairly common on the fruit of several varieties of bell and hot peppers in plantings at Experiment in 1945 and 1946. The fungus always appeared to have entered the fruit through either blossom-end rot or sunscald spots (Fig. 2, B). Once established in the dead tissue of these spots, however, it spread into the surrounding living tissue and rotted the fruit. This fruit rot has been duplicated by artificial inoculations in wounds (Fig. 2, D).

Tomato. Acting as a wound parasite, *Diaporthe phaseolorum* var. *sojae* is able to cause a rot of tomato fruit. The lesions are circular, slightly depressed, water-soaked areas (Fig. 2, E, F). The infected tissue is discolored. On green fruits it is dark green; on ripe fruits it is dark red. Ultimately the fruit is reduced to a soft-rotted, wrinkled, blackened mass with pycnidia scattered over its surface. Natural infection of tomato in the field was observed once in 1946 on a few fruits in a home garden at Experiment (Fig. 2, F). The fungus had entered through sunscald spots. A similar rot has easily been produced, however, by inoculating the fungus into wounds on green or ripe fruits (Fig. 2, E).

Okra. In December, 1945, a single pod of okra infected with *Diaporthe phaseolorum* var. *sojae* was found near Experiment. The pycnidia of the fungus were aggregated to form a small black patch on the surface of the dead pod (Fig. 2, C). This pod had been left hanging on the plant in the field after maturity. The fungus appeared merely to have invaded the pod after it had matured and died naturally.

Onion and Garlic. Pycnidia of *Diaporthe phaseolorum* var. *sojae* were found on dead tips of leaves of onion and garlic plants in home gardens at Experiment in the spring of 1946. Only a few leaves were infected.

ETIOLOGY

Morphology. The pycnidium of *Diaporthe phaseolorum* var. *sojae* originates beneath the host epidermis as a stromatic mass of pseudoparenchymatous cells. Across the middle of this stroma a layer of fertile cells develops. These cells become elongated to form short, simple, unicellular conidiophores. As a result of their growth the stroma is split to produce a slit-like cavity into which the conidiophores project from all sides. Pycnospores are abstricted singly from the tips of the conidiophores and fill the cavity. As the pycnospores develop, the cells which produced them disintegrate, and cells of the stroma beneath them in turn become fertile. The cavity is thus gradually enlarged until only the outer layers of the stroma remain to form the wall of the pycnidium. The wall, therefore, varies in thickness, becoming gradually thinner as the pycnidium matures. At maturity the wall is composed of a layer of three to six brown, thick-walled, flattened cells surrounding the central cavity. Toward the apex the wall is thicker and composed of isodiametric cells, forming a short, rounded or conical, ostiolate beak. The

cavity is lined with a layer two to four cells thick of hyaline, isodiametric cells from which the condiophores arise. Pycnidia usually contain only a single cavity. Pycnidia with two or three cavities occur commonly, however, and pycnidia containing five to ten cavities may be found occasionally. Often the walls between two cavities are disintegrated, forming a single cavity which is, however, provided with two separate ostioles.

In surface view pycnidia on stems are elliptical with the longer axis parallel to the axis of the stem. On leaves and pods they are round to oval. In sections the pycnidia on stems and pods are lenticular, while on leaves they are more nearly spherical. Pycnidia varied greatly in size on the same host, being much smaller on leaves than on stems and pods. There seemed, however, to be no difference in size of pycnidia correlated with difference in host. For example, on snap-bean leaves pycnidia measured 95–245 × 82–163 μ (mean 148 × 122 μ), on snap-bean pods 163–408 × 136–340 μ (mean 283 × 236 μ), and on soybean pods 190–490 × 177–313 μ (mean 275 × 246 μ).

Pycnospores of *Diaporthe phaseolorum* var. *sojae* are 1-celled, hyaline, elliptical, and rounded at both ends. They contain a single central nucleus and usually a single conspicuous oil globule in either end of the spore. Occasionally three to four oil globules are present. There was little difference in size of pycnospores from different parts of the same host or from different hosts.

The stylospores differ from pycnospores in that they are long, filiform, usually curved spores lacking the conspicuous oil globules which are characteristic of the pycnospores. Spores intermediate between typical stylospores and typical pycnospores occur frequently. Stylospores have been found only a few times and then usually in late fall. They have been found only on soybean, cowpea, and lima bean. They occur so rarely, however, that this is considered to be merely a matter of chance.

Pycnidia of *Diaporthe phaseolorum* are similar in structure and dimensions to those of *D. phaseolorum* var. *sojae* (Table 1). On lima-bean stems, pycnidia of *D. phaseolorum* measured 136–490 × 122–326 μ (mean 242 × 201 μ), those of the variety *sojae* 136–408 × 109–299 μ (mean 245 × 177 μ). Pycnospores of *D. phaseolorum* are longer and wider both in mean and maximum dimensions than those of the variety *sojae* (Table 1). The difference in size is slight, however, and sometimes samples of pycnospores of *D. phaseolorum* approach samples of pycnospores of the variety closely in size. For example, the lowest measurements recorded for samples of *D. phaseolorum* pycnospores were 6.0–11.3 × 2.7–3.5 μ (mean 8.0 × 3.0 μ); the highest measurements for samples of pycnospores of the variety *sojae* were 6.0–9.5 × 2.3–3.3 μ (mean 7.8 × 3.0 μ). Stylospores of *D. phaseolorum* are distinctly larger than those of the variety *sojae* (Table 1).

Perithecia of *Diaporthe phaseolorum* var. *sojae* were found in the field on over-wintered stems of soybean, cowpea, lima bean, and snap bean (Fig. 1, B). Perithecia developed also within two months on infected stems of soybean and cowpea brought into the laboratory in August and in October and placed in beakers partly filled with water.

TABLE 1.—*A comparison of the morphology of Diaporthe phaseolorum and D. phaseolorum var. sojae*

Structure	On host		In culture		
	D. phaseolorum[a]	*D. phaseolorum* var. *sojae*	*D. phaseolorum*	*D. phaseolorum* var. *sojae* (perithecial strains)	*D. phaseolorum* var. *sojae* (nonperithecial strains)
Pycnidia	136–639 × 122–354μ	95–598 × 82–408μ	Separate	In stroma	In stroma
Grand mean	(50) 267 × 230μ	(400) 240 × 185μ			
Variation in sub-means[b]	242–332 × 201–259μ	148–297 × 122–246μ			
Pycnospores	5.3–12.0 × 2.5–4.5μ	4.5–9.8 × 1.1–3.9μ	5.3–13.5 × 2.3–6.0μ	4.5–9.0 × 1.8–3.6μ	4.5–9.0 × 1.6–3.3μ
Grand mean	(175) 8.6 × 3.1μ	(650) 7.3 × 2.7μ	(100) 7.9 × 3.1μ	(525) 6.6 × 2.7μ	(600) 6.4 × 2.3μ
Variation in sub-means	7.9–9.2 × 2.7–3.6μ	6.4–8.0 × 1.8–3.0μ	7.4–9.2 × 3.0–3.4μ	6.2–7.4 × 2.4–2.9μ	5.8–7.2 × 1.9–2.7μ
Stylospores	14.0–30.8 × 1.4μ	9.0–21.0 × 0.8–1.8μ	15.0–61.5 × 1.2–2.3μ	9.8–22.6 × 0.7–1.6μ	None
Grand mean	(25) 21.4 × 1.4μ	(59) 14.3 × 1.3μ	(100) 34.1 × 1.5μ	(525) 15.8 × 1.3μ	
Variation in sub-means		12.9–15.3 × 1.3–1.4μ	27.0–39.8 × 1.4–1.5μ	12.9–19.7 × 1.1–1.4μ	
Perithecia	158–356μ	163–272 × 190–340μ	None	In stroma	None
Grand mean	252μ	(100) 218 × 254μ	do		do
Variation in sub-means		199–255 × 243–291μ	do		do
Asci	28.0–46.2 × 5.2–8.0μ	28.0–44.8 × 7.8–10.6μ	do		do
Grand mean	37.4 × 6.7μ	(75) 38.4 × 8.6μ	do		do
Variation in sub-means		38.3–38.5 × 8.6μ	do		do
Ascospores	6.4–12.0 × 2.3–4.0μ	9.0–13.5 × 3.0–4.8μ	do	8.0–13.5 × 2.9–5.3μ	do
Grand mean	9.5 × 2.9μ	(100) 11.1 × 3.7μ	do	(525) 10.2 × 3.4μ	do
Variation in sub-means		10.8–11.6 × 3.5–4.0μ	do	9.6–12.0 × 3.1–4.2μ	do

[a] Measurements of perithecia, asci, and ascospores of *D. phaseolorum* are from Harter (4).

[b] Sub-means are means of samples of 25 measurements each. The number of measurements from which each grand mean is derived is indicated in parentheses preceding the mean.

The perithecium originates from a coiled, multicellular ascogonium which develops from the mycelium in the inner cortex of the host. The ascogonium becomes enveloped by a spherical mass of hyphae which form the wall of the perithecium. The interior of the perithecium is filled with a mass of large, hyaline, pseudoparenchymatous cells. The ascogenous hyphae are embedded in the center of this pseudoparenchyma, and from them the asci arise. As the asci expand, the pseudoparenchyma is crushed and disintegrated, and the asci push down into the base of the perithecium. At the time the asci begin to develop, hyphae in the apex of the perithecial wall grow upward in a column, forming the ostiolate neck of the perithecium. The neck extends tortuously through the cortex and epidermis to the exterior of the host stem (Fig. 1, A, B). Development of the perithecium appears to be essentially similar to that of *Ceratostomella adiposum* (Butl.) Sartoris (13). The perithecia are embedded singly in the host tissues. The limits of growth of the mycelium are marked by a black line in the host tissue.

Mature perithecia are roughly spherical but are usually flattened at the base so that they are more wide than high. The walls surround a central cavity filled across the lower two-thirds with a mass of aparaphysate asci. The asci are clavate, and each contains eight ascospores. They break loose at the base and their walls disintegrate, liberating the spores which, embedded in a viscous fluid, ooze out through the ostiole. The ascospores are hyaline, uniseptate, elliptical, rounded at either end, and slightly constricted at the septum. Each cell of the ascospore is uninucleate and contains one large and one small oil globule. The length of the perithecial neck varies, depending upon the moisture conditions under which it develops. This was demonstrated in culture on soybean petioles in culture tubes partially filled with water. Toward the moist base of the petiole the necks were long and coiled, toward the drier upper portion they became progressively shorter (Fig. 1, A). There was little difference in the perithecial stage of the fungus on the four different hosts.

No perithecia of *Diaporthe phaseolorum* have been examined. From Harter's (4) description, however, it is apparent that they are essentially similar to perithecia of the variety *sojae*. They differ slightly in width of asci and in size of ascospores, those of *D. phaseolorum* being the smaller (Table 1).

Growth in culture. *Diaporthe phaseolorum* var. *sojae* was isolated from all recorded hosts, with the exception of *Strophostyles helvola*. Of these isolates, 45 have been studied in culture. They were obtained from the vicinity of Experiment in middle Georgia and from the vicinities of Arabi, Tifton, Albany, and Leesburg in south Georgia. Dr. L. L. Harter kindly furnished an isolate of *D. phaseolorum* obtained from lima bean in Maryland. Material of this fungus was not found in Georgia until 1946. The cultures were maintained on three per cent malt agar. The fungi fruited more abundantly, however, on snap-bean stems and soybean petioles partially immersed in water in culture tubes and on cornmeal mush. Perithecia

were produced more readily in cool weather. In making comparisons of pycnidia and perithecia in culture, the isolates were grown at room temperature on cornmeal mush in 250-cc. flasks.

Isolates of *Diaporthe phaseolorum* var. *sojae* formed a white, flocculent, rapidly growing mycelium. There was a tendency for the aerial hyphae to become loosely united in slender strands. The mycelium of *D. phaseolorum* was similar but was more closely appressed, and the hyphae did not form strands. It also grew more slowly. The most striking differences between the two fungi appeared, however, in the production of fructifications. As previously described by Harter (4), colonies of *D. phaseolorum* produced only pycnidia in culture. Perithecia were never formed on any medium employed. The pycnidia were scattered singly over the surface of the medium enmeshed in the mycelial weft. They were composed of a thin wall of loosely compacted hyphae surrounding a large central cavity in which pycnospores and later stylospores were produced abundantly. The spores oozed from the pycnidia and collected in large yellowish or orange droplets at the mouths of the ostioles (Fig. 2, G, left).

On cornmeal mush and on agar media pycnidia of *Diaporthe phaseolorum* var. *sojae* were produced in groups in conspicuous, black, pulvinate stromata formed on the mycelium (Fig. 2, G, right). The stromata were 1–9 mm. in diameter and often were produced so abundantly that they almost covered the white mycelium with a black crust. The interior of the stroma was plectenchymatous, the peripheral portions were pseudoparenchymatous. Pseudoparenchymatous pycnidia were embedded in the surface of the stroma with their beaks more or less protruding. Pycnospores were produced abundantly in most cultures, but they rarely oozed from the ostioles in conspicuous globules as in *D. phaseolorum*. Although pycnospores of both fungi in culture were smaller than those produced in the field, the same relative difference between them was maintained. The difference in size of stylospores was greater in culture than in the field (Table 1). In some strains perithecia were produced later in the same stromata beneath the pycnidia. It is interesting to note that, while on the host stems in nature the perithecia are embedded separately in the host tissue, in culture they are grouped in a diatrypoid stroma.

Although all isolates of *Diaporthe phaseolorum* var. *sojae* were alike in the production of stromata and thus distinct from isolates of *D. phaseolorum*, they varied widely among themselves. The various isolates were often recognizable individuals. Size of stromata varied greatly. Some isolates formed almost smooth or slightly botryose stromata; in others the stroma became echinulate through the production of long pycnidial beaks. Some were almost sterile; others produced pycnospores abundantly. As Lehman (8) found previously, some isolates produced only pycnospores; others produced stylospores as well. Perithecia were developed by only about half of the isolates. It was soon apparent that there was a correlation among some of these characters. All isolates could be divided into two

major groups, the perithecial strains and the nonperithecial strains (Table 1). The perithecial strains always produced perithecia on all media, formed stylospores, and showed a tendency to form the echinulate type of stroma. The nonperithecial strains never produced perithecia under any conditions, never formed stylospores on the usual media, and tended to form smooth or botryose stromata. Many of them produced pycnospores only sparingly. Of the 45 isolates tested, 21 were perithecial strains and 24 were nonperithecial strains. Perithecial strains predominated among isolates from middle Georgia. Only 12 of the 31 isolates from this area were nonperithecial, while 12 of the 14 isolates from south Georgia were nonperithecial strains.

The failure of many of these isolates to form perithecia probably cannot be attributed to the existence of different sexual strains, because Tucker (16) has demonstrated that *Diaporthe phaseolorum* var. *sojae* (referred merely to the *D. phaseolorum* complex) is homothallic. The production of both perithecial and nonperithecial strains seems to be common among species of *Diaporthe*. It has also been reported by Leach (7) in *Ceratostomella ips* Rumb. Leach was unable to induce the formation of perithecia in *C. ips* by mating nonperithecial strains. In *D. phaseolorum* var. *sojae* mating of two nonperithecial strains from Experiment and two from Tifton likewise failed to induce formation of perithicia. Nonperithecial strains possibly are not incapable of producing perithecia. They merely have not done so under cultural conditions.

By using the method of Nitimargi (10) it has been possible to demonstrate that at least some of the nonperithecial strains are capable of producing stylospores although they do not produce them on ordinary media. Two lots of medium were prepared. Medium A contained 2 gm. sucrose, 2 gm. asparagin, 0.75 gm. magnesium sulfate, 1.25 gm. potassium phosphate, 15 gm. agar, and 1,000 cc. water. Medium B was the same except that the amount of sucrose was increased to 128 gm. Eight tubes of each medium were inoculated with each of three perithecial and five nonperithecial strains. Half of the resulting cultures were kept in total darkness, the other half were exposed to normal daylight. Since all isolates fruited poorly on both media, the experiment was continued for three months. Of the cultures kept in total darkness none of the isolates on medium B fruited and only one of medium A formed pycnospores. This was a nonperithecial strain, and it did not form stylospores. The results from cultures exposed to daylight were as follows: on medium A one of the three perithecial strains produced pycnospores and stylospores, and three of the five nonperithecial strains produced pycnospores but no stylospores. On medium B one of the perithecial strains and two of the nonperithecial strains produced both pycnospores and stylospores. One of the nonperithecial strains produced only a few stylospores, but the other produced them in the ratio of one stylospore to four pycnospores. Measurements of spores from this culture were as follows: pycnospores, 5.3–7.7 × 2.0–2.5 μ (mean 6.6 × 2.3 μ); stylo-

spores, 9.3–26.6 × 0.8–1.3 μ (mean 21.4 × 1.1 μ). These measurements are close to those of spores produced normally by perithecial strains (Table 1).

Contrary to results obtained by Lehman (8), exposure to light was found unnecessary for the formation of pycnidia, although it did stimulate their production. Fresh transfers of the isolates to be tested were placed in total darkness for five days. Flasks of cornmeal mush and tubes of soybean petioles were inoculated from these cultures. They were then divided into two lots. One lot was exposed to light from a north window; the other was kept in total darkness for one month. The isolate of *Diaporthe phaseolorum* formed pycnospores and stylospores in darkness as well as when exposed to light. Of the 17 isolates of *D. phaseolorum* var. *sojae* tested, only four failed to form pycnospores in darkness, and these were nonperithecial strains which formed pycnospores poorly when exposed to light. The perithecial strains produced stylospores and perithecia also in total darkness. All isolates, however, fruited more abundantly when exposed to light.

Inoculations. Inoculations with pycnospores from cultures of various isolates of *Diaporthe phaseolorum* and *D. phaseolorum* var. *sojae* were made on snap bean, cowpea, lima bean, soybean, peanut, tomato, and pepper. The plants were grown in sterile soil in 8-inch pots with one to four plants in each pot. Approximately half of the plants were wounded on stems and pods with a razor blade. Pycnospore suspensions were then applied to all parts of the plants. After the inoculum had dried, the plants were placed in a moist chamber for 48 hours. They were then returned to benches in the greenhouse and kept under observation for at least two months. Controls run in all experiments were treated similarly except for the fact that they were not inoculated. Pepper and tomato fruits were inoculated in the field by inserting mycelium in wounds on green and ripe fruits that had been surface sterilized with 95 per cent alcohol. The wounds were then covered with adhesive tape. Inoculations were made on picked fruit by inserting mycelium in wounds on fruit that had been surface sterilized with mercuric chloride solution and placed in large, sterile culture dishes. Sterile agar was placed in wounds in control fruit. The results of these inoculations are presented in table 2.

Diaporthe phaseolorum infected only lima bean. Characteristic lesions, as described by Harter (4), appeared on the immature pods at the end of one week and were well developed, killing the pods, two weeks after the inoculations were made. Leaf infections were not obtained. The fungus entered the peduncles from infected pods and spread down the branches of the plants only after they became senescent. A higher percentage of infection would have been obtained except for the fact that on some plants the pods were so nearly mature that they began to dry before lesions developed on them. Inoculations on half-grown pods were always successful. Infection took place through the nonwounded epidermis more readily than through wounds. *D. phaseolorum* did not produce lesions on any other plants and never fruited on them after they had died from other causes.

It did not infect either pepper or tomato fruits even when inoculated into wounds.

Diaporthe phaseolorum var. *sojae* never infected seedling plants and never produced lesions on mature plants of any host with the exception of pepper and tomato. At the end of two months it was fruiting on many dead stems of all hosts (Fig. 1, C, I). By this time many of the plants were senescent or had died naturally, as might be expected since they were fully mature when they were inoculated. It is significant that the number of dead plants usually exceeded the number of infected plants and that often a greater number of control plants than of inoculated plants was dead. Infection with *Macrophomina phaseoli*, resulting presumably from wind-borne pycnospores, caused the death of some plants. After the control plants and some of the plants inoculated with *D. phaseolorum* died, *D. phaseolorum* var. *sojae* fruited on them. Apparently it spread readily from inoculated plants that had died earlier. Infection of wounded green or ripe pepper (Fig. 2, D) and tomato (Fig. 2, E) fruits with *D. phaseolorum* var. *sojae* was consistently obtained. Infection produced a fruit rot similar to the rot of these fruits resulting from natural infection in the field. The fungus did not, however, invade ripe, nonwounded tomato fruits when they were placed in contact with rotted fruits in a culture dish.

Because Lehman (8) had reported successful inoculations on soybean with *Diaporthe phaseolorum* var. *sojae*, some additional inoculations were made on this host. The host material was large, fully mature soybean plants grown singly in 8-inch pots. These were divided into two lots of which one was inoculated with a suspension of pycnospores from soybean stems naturally infected in the field, while the other lot was retained as controls. Half of the plants in each lot were wounded on stems and pods. The plants were placed in a moist chamber for 48 hours and then removed to a cloth house where they were exposed to a fine spray of water every night for two weeks and were kept moist for two weeks thereafter. Results at the end of one month were as follows: of six wounded, inoculated plants six were infected; of six nonwounded, inoculated plants two were infected; of six wounded, control plants none was infected; and of six nonwounded, control plants none was infected. At this time all plants, both inoculated and control, were dead. *Glomerella glycines* (Hori) Lehman and Wolf, accompanied by *Fusarium sp.* and various molds, was fruiting on all of these plants. Under these conditions of prolonged high humidity, some infection with *D. phaseolorum* var. *sojae* was obtained; but at the same time various saprophytic fungi also fruited on the same plants. Since the control plants died as soon as the inoculated plants, it is impossible to conclude that *D. phaseolorum* var. *sojae* was responsible for the death of the plants on which it fruited.

Since *Diaporthe phaseolorum* var. *sojae* was isolated from snap-bean plants killed by root rot, inoculations were made to determine whether it might infect seedlings through the soil. In the first test, pots of sterile soil

TABLE 2.—*Results of inoculations with Diaporthe phaseolorum and D. phaseolorum var. sojae on various crop plants*

Host	Plants inoculated	Results			
		One month		Two months	
		Plants dead	Plants infected	Plants dead	Plants infected
	number	*per cent*	*per cent*	*per cent*	*per cent*
Inoculated with *D. phaseolorum* var. *sojae*					
Snap bean					
Seedling	4	0	0	0	0
Mature	76	3.9	1.3	15.8[a]	13.2
Cowpea					
Seedling	8	0	0	0	0
Mature	53	3.8	1.9	71.7[b]	34.0
Lima bean					
Seedling	1	0	0	0	0
Mature	54	0	1.9	16.7[c]	7.4
Soybean					
Seedling	21	0	0	0	0
Mature	33	0	0	57.6	57.6
Peanut					
Mature	4	0	0	0	0
Tomato					
Seedling	8	0	0	0	0
Fruit	20	.....	25.0	.....	.....
Picked fruit					
Green	22	.....	63.9	.....	.....
Ripe	7	.....	100.0	.....	.....
Pepper					
Seedling	10	0	0	0	0
Mature	10	0	0	0	0
Fruit	21	.....	81.0	.....	.....
Picked fruit	22	.....	0	.....	.....
Inoculated with *D. phaseolorum*					
Snap bean					
Seedling	4	0	0	0	0
Mature	19	10.5	0	21.0	0[d]
Cowpea					
Seedling	8	0	0	0	0
Mature	28	0	0	7.1	0[e]
Lima bean					
Seedling	6	0	0	0	0
Mature	31	0	54.8	0	54.8
Soybean					
Seedling	14	0	0	0	0
Mature	16	0	0	50.0	0[f]
Peanut					
Mature	2	0	0	0	0
Tomato					
Seedling	4	0	0	0	0
Fruit	10	.....	0	.....	.....
Picked fruit					
Green	12	.....	0	.....	.....
Ripe	3	.....	0	.....	.....
Pepper					
Seedling	5	0	0	0	0
Mature	5	0	0	0	0
Fruit	10	.....	0	.....	.....
Picked fruit	11	.....	0	.....	.....

TABLE 2. (*Continued*)

Host	Plants inoculated	Results			
		One month		Two months	
		Plants dead	Plants infected	Plants dead	Plants infected
	number	*per cent*	*per cent*	*per cent*	*per cent*
Noninoculated control					
Snap bean					
Seedling	4	0	0	0	0
Mature	19	0	0	42.1	42.1[f]
Cowpea					
Seedling	4	0	0	0	0
Mature	19	21.0	0	57.9	15.8[g]
Lima bean					
Seedling	2	0	0	0	0
Mature	15	0	0	0	0
Soybean					
Seedling	7	0	0	0	0
Mature	14	0	0	78.6	21.4[g]
Peanut					
Mature	2	0	0	0	0
Tomato					
Seedling	4	0	0	0	0
Fruit	10	.....	0	.....	.....
Picked fruit					
Green	7	.....	0	.....	.....
Ripe	3	.....	0	.....	.....
Pepper					
Seedling	5	0	0	0	0
Mature	5	0	0	0	0
Fruit	12	.....	16.7[g]	.....	.....
Picked fruit	11	.....	0	.....	.....

[a] Four plants killed by *Macrophomina phaseoli*.
[b] Ten plants killed by *M. phaseoli*.
[c] All killed by *M. phaseoli*.
[d] Two plants infected with *D. phaseolorum* var. *sojae*.
[e] One plant infected with *D. phaseolorum* var. *sojae*.
[f] Eight plants infected with *D. phaseolorum* var. *sojae*.
[g] All infected with *D. phaseolorum* var. *sojae*.

were inoculated with a culture of the fungus grown on sterile oats. Sterile oats were mixed with the soil in control pots. Of 40 snap-bean seeds planted in inoculated soil, 37 emerged. At the end of two months 33 were healthy mature plants. Four plants were killed by a *Pythium sp.* Of 20 seeds planted in control pots, 17 emerged and 16 plants survived to maturity. One was killed by *Pythium sp.* In the second test, seedling snap-bean plants growing in sterile soil in 8-inch pots were inoculated by digging into the soil around the plants and placing oats, on which the fungus was growing, in contact with the roots and hypocotyls. Sterile oats were placed around the control plants. Of 28 inoculated plants, 22 were healthy two months later. Six had been killed by *Pythium sp.* Of 13 control plants, 9 survived to maturity, 4 being killed by *Pythium sp.* *D. phaseolorum* var. *sojae* never fruited on any of these plants.

DISCUSSION

Host range. *Diaporthe phaseolorum* is limited to lima bean, the host on which it was orginally described. Inoculation experiments have not confirmed reports of its occurrence on any other host. Tucker (16) referred the fungus causing a fruit rot of pepper in Texas merely to the group that Wehmeyer (17) included in *D. phaseolorum*. Because of the dimensions of pycnospores and stylospores and the production of stromata and perithecia in culture, it is evident that this fungus was *D. phaseolorum* var. *sojae*. It likewise seems certain that the fungus Bratley and Wiant (2) found on tomatoes in New York markets was this variety. Hopkins (5) stated only that the fungus he found on tomato in Rhodesia resembled *D. phaseolorum*. It is possible that this also was the variety *sojae*, but too little information was furnished to determine its identity. Seymour's (14) report of *D. phaseolorum* on snap bean apparently was a mistake. No other reference to its occurrence on this host has been found in the literature. A specimen collected by W. D. Moore at Cairo, Georgia, June 20, 1931, and labelled *D. phaseolorum* on *Phaseolus vulgaris* was obtained from the Bureau of Plant Industry Mycological Collections. This proved to be *Macrophomina phaseoli* on lima-bean stems.

On the other hand, *Diaporthe phaseolorum* var. *sojae* has been found on 13 different hosts, and it is probable that this list of hosts will be extended. Although there were variations among specimens of the fungus on different hosts and on different host parts and among various isolates of the fungus in culture, they all seem referable to *D. phaseolorum* var. *sojae*.

Pathogenicity and importance. My inoculations show, as did those of Harter (4), that *Diaporthe phaseolorum* is pathogenic to lima bean. It may enter non-wounded immature pods and produce characteristic lesions on them within two weeks. The inoculations have not shown that *D. phaseolorum* var. *sojae* is a primary pathogen on any of its hosts. It may cause a rot when inoculated into wounds in pepper and tomato fruits. Under natural conditions, however, it always appears to be secondary, entering the fruits through blossom-end rot or sunscald spots. On all other hosts this fungus seems to be merely a vigorous saprophyte, quickly fruiting on plants or plant parts killed or greatly weakened by other organisms or by adverse growing conditions. It may then appear to have caused the death of the plants on which it is fruiting, but careful study will usually reveal that other environmental factors are primarily responsible. There was no evidence that soybean is more susceptible to infection with *D. phaseolorum* var. *sojae* than any other hosts on which it occurs, such as snap bean and cowpea. Lehman (8) obtained infection of soybean for the most part by keeping inoculated plants covered with bell jars for long periods. Furthermore, his results were not checked against control plants. Under similar conditions of high humidity for a long period, *D. phaseolorum* var. *sojae* has been induced to fruit on pods and stems of dying soybean plants. This can hardly be considered proof of the pathogenicity of *D. phaseolorum* var. *sojae*, however, because under these conditions various saprophytic fungi

also fruited on both inoculated and control plants. This fungus must, therefore, have little influence upon crop yields. Under favorable conditions it might cause some crop loss through decay of seed, but such losses have not yet been found in Georgia.

Taxonomy. Because of the differences in pathogenicity and in host range between *Diaporthe phaseolorum* and *D. phaseolorum* var. *sojae*, it is desirable that these two fungi be recognized taxonomically. The question remaining is what taxonomic category should be employed, and this must be answered by a consideration of comparative morphology. On the host no differences in structure or dimensions of pycnidia or perithecia of the two fungi have been found. Stylospores of *D. phaseolorum* were much larger than those of the variety *sojae*, but they were found so rarely in field material of either fungus that this distinction is of little practical importance. Pycnospores of *D. phaseolorum* were slightly larger, exceeding those of the variety by 2.2 μ in maximum length and by 1.3 μ in mean length. The differences in maximum and in mean widths were 0.6 μ and 0.4 μ, respectively (Table 1). On the other hand, the asci of the variety were slightly wider and the ascospores slightly larger than those of the species, the difference in ascospore dimensions being in both maximum and mean length 1.5 μ and in both maximum and mean width 0.8 μ. In culture the two fungi are readily distinguished because of the production of pycnidia in pulvinate stromata in cultures of *D. phaseolorum* var. *sojae*. The difference in size of stylospores in culture is obvious (Table 1), but more than half of the isolates of the variety did not produce stylospores at all. *D. phaseolorum* did not form perithecia in culture, but neither did many isolates of *D. phaseolorum* var. *sojae*. Although the pycnospores produced in culture by both fungi were smaller than those produced in nature, the same relative difference was maintained (Table 1). Nevertheless, the difference was slight, and means of small samples of pycnospores of the two fungi occasionally overlapped. Also differences in size of pycnospores of each fungus in field material and in culture were almost as great as the differences between the two fungi under the same conditions.

Although these differences in spore size are generally sufficient to distinguish the two fungi, positive determination of *Diaporthe phaseolorum* and *Diaporthe phaseolorum* var. *sojae* upon the basis of spore measurements might not always be possible. Positive separation of them depends primarily upon differences in pathogenicity and in appearance in culture. Since these differences are accompanied by slight differences in morphology, *D. phaseolorum* var. *sojae* should be considered a well established variety. These differences might be considered sufficient to warrant specific rank. It seems best, however, to follow Wehmeyer's (17) treatment of the genus *Diaporthe*, maintaining this fungus as a variety of *D. phaseolorum*.

SUMMARY

Although *Diaporthe phaseolorum* (C. & E.) Sacc. has been reported on tomato, pepper, and snap bean, inoculations have shown that it is limited

to lima bean, the host on which it was originally described. On lima bean it is definitely pathogenic, entering through the non-wounded epidermis of immature pods and producing characteristic lesions.

Diaporthe phaseolorum var. *sojae* (Lehman) Wehmeyer has been found on the following hosts: soybean, snap bean, cowpea, lima bean, peanut, lupine, lespedeza, *Strophostyles helvola*, tomato, pepper, okra, onion, and garlic. Acting as a wound parasite, it is capable of causing a rot of pepper and tomato fruits. It enters these fruits through blossom-end rot or sunscald spots. There is no evidence from observations or inoculations that it is pathogenic to any other host. It appears to be merely a vigorous saprophyte that may quickly fruit upon plants killed by other organisms or by adverse growing conditions. It might cause crop losses through the decay of seed of plants such as soybean, cowpea, snap bean, and lima bean; but it has not been observed to do so in Georgia.

Diaporthe phaseolorum var. *sojae* differs from *D. phaseolorum* primarily in pathogenicity and in the production in culture of pycnidia, and in some strains perithecia, in conspicuous diatrypoid stromata. Stylospores of the var. *sojae* are smaller than those of *D. phaseolorum*, but these spores are rarely produced in nature by either fungus. The ascospores of the variety are slightly larger and the pycnospores slightly smaller than those of the species. Because the morphological differences are slight, the classification of this fungus as a variety of *D. phaseolorum* has been accepted.

In culture, isolates of *Diaporthe phaseolorum* var. *sojae* may be divided into perithecial and nonperithecial strains. Perithecial strains produce pycnospores, stylospores, and perithecia and tend to form echinulate stromata. Nonperithecial strains never produce either stylospores or perithecia and tend to form smooth or botryose stromata. They often produce pycnospores only sparingly. Within each strain individual isolates vary in size and shape of stromata, in fertility, and in general appearance. Of 45 isolates obtained from various hosts in Georgia, 21 were perithecial strains, 24 were nonperithecial strains.

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SEEDLING BLIGHT AND ROOT ROT OF FLAX IN WASHINGTON[1]

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INTRODUCTION

Many complaints of poor stands of flax, even when planted under apparently favorable conditions, have been received from Washington growers. Moreover, workers in the Washington Agricultural Experiment Station, at Pullman, in their varietal yield trials found that samples of seed gave different emergence counts depending on the source of the seed. Workers in Europe and North America have shown that species of *Pythium*, *Phytophthora*, *Asterocystis*, *Ophiobolus*, *Helminthosporium*, *Botrytis*, *Alternaria*, *Cladosporium*, *Colletotrichum*, *Rhizoctonia*, and *Thielavia* were involved in the so-called "soil sickness" complex. The literature on the subject was reviewed by Schilling (4) and Tervet (6). Flor (1) studied *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hansen (*Fusarium lini* Bolley) and found this organism to be mainly responsible for soil sickness in North Dakota. It has been shown also that flax with injured seed coats gave poor emergence (2, 3, 5). Treatment of seed with protective dusts has improved stands, particularly when cracked seed was used (2, 3, 5). Seed treatment was of little value in North Dakota, since the wilt organism was primarily responsible for flax injury there (1). Investigations of the causes of poor stands of flax in Washington and of the effectiveness of seed treatments for the improvement of stands were initiated in 1941.

METHODS

To determine the organisms responsible for seed decay and seedling blight of flax, isolations were made from the surface of seed and from diseased roots and stems of seedlings from seed grown in Washington. Whole seeds and bits of diseased root and stem tissue were planted on potato-dextrose agar in Petri dishes in the usual manner. The resulting isolates which were to be tested for pathogenicity were purified by making successive transfers of hyphal tips, or of single spores where possible. Many isolates were eliminated after preliminary tests on the flax variety Bison. All isolates were identified as to genus. Those which proved pathogenic in preliminary tests were identified as to species. The fungi to be tested for pathogenicity were increased in Richard's solution in Erlenmeyer flasks and the mycelial mats were cut into small strips for addition to 4-inch pots of steamed soil. Twenty-five flax seeds were sown in each of these pots and each experiment was replicated four times in the greenhouse.

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[2] Formerly Research Assistant and Chairman, Division of Plant Pathology, respectively.

The writers are grateful to Dr. J. G. Harrar, formerly Chairman, Division of Plant Pathology, under whose direction the work was initiated.

Determination of percentage of cracked seed and selection of cracked seed for study were done with the aid of a dissection microscope.

The value of seed treatments in improving stands was determined in the greenhouse for five varieties of flax, namely: Bison, Rio, Redwing, Viking, and Zenith. Machine-threshed and hand-threshed lots of seed were treated with New Improved Ceresan (5 per cent ethyl mercuric phosphate), Micronized Sulphur, Semesan (30 per cent hydroxymercuri chlorophenol), Spergon (tetrachloro-parabenzoquinone), Du Bay 1205–FF (50 per cent tetramethyl thiuramdisulfide), Yellow Cuprocide (83 per cent yellow cuprous oxide), and Micronized Copper at the rate of 0.25 per cent by weight and planted in the greenhouse bench. Stands were determined by an actual count of seedlings soon after the first true leaves unfolded. Similar tests were made with the variety Bison, threshed by hand and by machine at low and high cylinder speeds,[3] planted in the field.

FUNGI ISOLATED FROM SEED AND SEEDLINGS

Fungi isolated from flax seed were species of *Penicillium, Alternaria, Aspergillus, Rhizopus,* and *Trichoderma.* Of these *Penicillium spp.* were isolated most frequently. Preliminary tests of these organisms in steamed soil failed to indicate marked pathogenicity for flax. Cultures were retained for studies with cracked seed.

Fungi isolated from flax seedlings grown in the field at Pullman were principally *Fusarium spp.* and *Alternaria spp. Fusarium* was isolated from such material 76 times while *Alternaria* was isolated only six times. Preliminary study showed the isolates of *Fusarium* to be similar and pathogenic although some were more strongly pathogenic than others. Isolates of *Alternaria* again were not pathogenic.

Isolation of fungi from diseased seedlings produced in pans of soil in the Seed Laboratory yielded *Fusarium* and *Rhizopus. Rhizopus* proved nonpathogenic and was discarded. Isolates of *Fusarium* were pathogenic and similar to those obtained from field-grown seedlings.

In the course of preliminary tests of the pathogenicity of isolates and

TABLE 1.—*Frequency of isolation and pathogenicity of most active isolates of Fusarium oxysporum and F. solani to flax in steamed soil as indicated by emergence and root and stem lesions on survivors*

Fungus	Isolates	Pathogenicity to flax	
		Emergence	Survivors with root and stem lesions
	Number	*Per cent*	*Per cent*
Fusarium oxysporum	159	27	70
F. solani	8	85	18
None	.....	89	1

[3] This seed was obtained from Dr. O. A. Vogel, Agronomist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture.

attendant comparison of colony characteristics and spores on potato-dextrose agar, it became evident that two pathogenic species had been obtained. These were identified as *Fusarium oxysporum* Schlecht and *F. solani* (Mart.) App. and Wr.[4] *F. oxysporum* was isolated from diseased seedlings 159 times while *F. solani* was isolated only eight times (Table 1). *F. oxysporum*, in later pathogenicity tests, also proved to be the more strongly pathogenic of the two. Differences in pathogenicity of three *Fusarium* isolates are shown in figure 1.

Isolates of *Fusarium oxysporum* obtained in other studies from roots of peas compared well in pathogenicity tests on flax with the isolates obtained from flax seedlings. Symptoms produced by this organism on flax consist of pre-emergence decay, damping-off, or, on older seedlings, pink to red or later brown, irregular lesions on root or stem, or both. These lesions may

FIG. 1. Emergence of Bison flax seedlings from 25 seeds sown in soil infested with 3 isolates of *Fusarium*. Check, at left, in steamed soil.

be small or involve the entire root and the base of the stem. Tests of the fungus on the wilt-susceptible variety Punjab failed to show yellowing, thickening of apical leaves, or wilting, characteristic of flax wilt, among the survivors. Symptoms of affected seedlings were alike in field soil and in pots of steamed soil to which *F. oxysporum* was added, but the incidence was higher in the latter.

SEED COAT INJURY AS A FACTOR IN SUSCEPTIBILITY TO FUNGUS ATTACK

Other workers have shown that sample lots of flax seed vary in amount of cracking. Machacek and Brown (2) found more cracking in samples from Western than from Eastern Canada. These workers also state that large-seeded varieties seemed more susceptible to injury than the small-seeded lots. Moore and Christensen (3) found that seed of Bolley's Golden, selection C.I. 976, grown in 31 localities in North America varied from 0 to 32 per cent cracked seed. They stated "the differences in amount of injury between seven varieties in a single locality were sometimes almost as great

[4] Identifications by Dr. W. C. Snyder, Associate Plant Pathologist, University of California.

TABLE 2.—*Percentage of cracked seed in flax varieties grown in Washington*[a]

Variety	Number of seed examined	Percentage of seed cracked
Bison	123	75
Viking	124	65
Rio	232	42
Redwing	189	15
Zenith	336	10

[a] Viking from 1940 crop; all others from 1941 crop.

as those between seed lots of a single variety grown at different localities." The fact that a sample of Bison flax from Minnesota gave better stands than a sample of Bison from the Palouse, although markedly pathogenic organisms were not present, made it seem reasonable to assume that cracked seed coats were responsible in part for flax seedling diseases in the Palouse.

In seed lots of several flax varieties grown in Washington 65 to 75 per cent of the seeds had at least slight cracks in the seed coat, as determined by examination under a dissecting microscope. In some cases as much as 50 per cent were severely damaged. Present data show that while there is a tendency for large-seeded varieties to be more severely damaged, size alone does not determine the degree of damage. The variety Rio, which has larger seeds than Bison, had considerably less cracking (Table 2). Severe cracking is not confined to the seed coat, but extends into the seed and frequently damages the embryo. The cotyledons may be broken or the radicle injured so as to prevent proper germination. Machacek and Brown (2), and Moore and Christensen (3), have shown that there is a relationship between cracking in a seed lot and percentage stand obtained.

Comparative tests were made of the attack on cracked and sound seed by isolates 1 and 2 of *Fusarium* and by the more common seed-borne organisms, *Penicillium* and *Alternaria* (Table 3). *Fusarium* isolate 2 was equally virulent on cracked and sound seed. The *Fusarium* isolate 1 was pathogenic to cracked seed but not to sound seed. These isolates were similar in culture and identified as *F. oxysporum*. No further studies of strain differences were made. *Penicillium* and *Alternaria*, though detrimental, were much less so than the Fusaria. The data definitely show that fungi ordinarily

TABLE 3.—*Seedling emergence from cracked and sound flax seed inoculated with isolates of Fusarium, Penicillium, and Alternaria*

Fungus	Number of plants emerged[a] from seeds with seed coat	
	Sound	Cracked
Fusarium oxysporum 1	94	54
F. oxysporum 2	35	32
Penicillium sp.	93	70
Alternaria sp.	94	76
None	95	88

[a] Mean of two tests with 100 seeds in each variation.

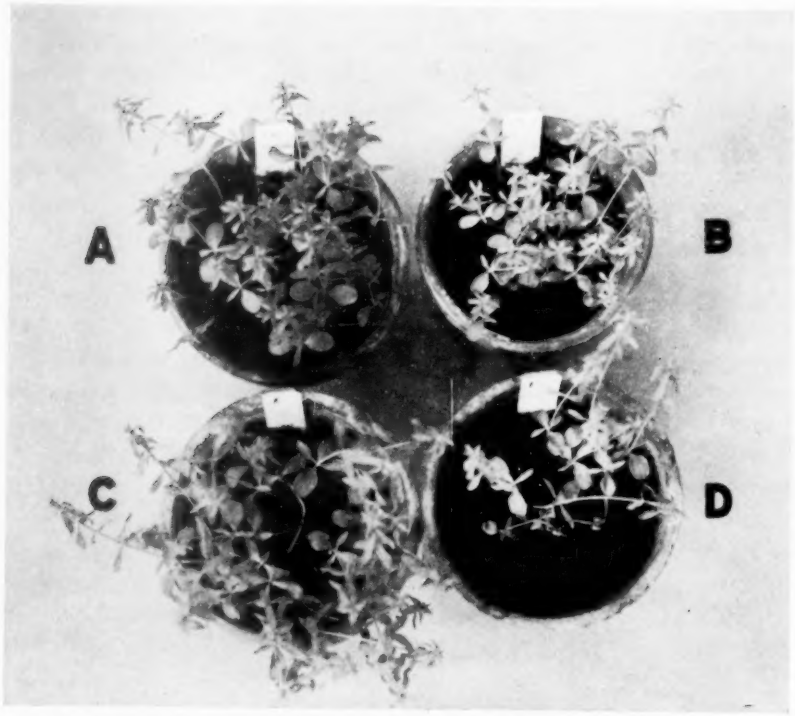

FIG. 2. Effect of broken seed coats of flax on pathogenicity of the otherwise avirulent *Fusarium* isolate 1. Pot A, sown with sound, uncracked seed and not inoculated; B, with cracked seed, not inoculated; C, with sound, uncracked seed, inoculated; D, with cracked seed, inoculated.

FIG. 3. Response of uninjured and injured flax seed to treatment with Spergon, in the greenhouse. Left to right—sound seed, treated; sound seed, not treated; cracked seed, treated; cracked seed, not treated.

considered as saprophytes can cause severe losses when seed-coats are injured. The influence of cracked seed is shown also in figures 2 and 3.

CONTROL OF DAMPING-OFF AND SEEDLING BLIGHT

Seed treatment has been a satisfactory means of controlling damping-off in Washington (5), Minnesota (3), and Canada (2). Seed of six flax varieties was threshed by hand or by machine at high cylinder speed. Seven lots treated with different fungicides and one check lot were sown in the greenhouse in 1942 (Table 4). The stands were greater for untreated hand

TABLE 4.—*Effect of seed treatment, applied at the rate of 0.25 per cent by weight, on emergence of flax seed threshed by hand and by machine at high cylinder speed. Greenhouse, 1942*

Variety	Type of threshing	Check[a]	Percentage increase in stand over check due to treatment with						
			New Improved Ceresan	Micronized sulphur	Semesan	Spergon	Du Bay 1205–FF	Yellow Cuprocide	Micronized copper
Bison	Hand	91	27	24	23	23	13	13	20
	Machine	20	360	−35	350	430	480	145	260
Redwing	Hand	96	22	27	16	25	13	19	25
	Machine	52	54	−12	110	87	88	83	81
Zenith	Hand	96	−3	0	13	9	23	16	−2
	Machine	48	102	8	110	83	113	78	106
No. 1046	Hand	95	−24	−3	−13	−5	−14	2	−17
	Machine	55	−24	−5	64	65	58	49	36
Viking	Hand	72	25	1	33	31	39	31	18
	Machine	19	26	111	275	442	400	153	146
Rio	Hand	97	23	15	−7	9	−1	4	15
	Machine	42	29	174	88	126	150	95	50

[a] The numbers indicate means of actual stands in four replications of 100 seeds.

threshed seed of all varieties than for machine threshed. This appears to confirm the statement that cracking is due chiefly to threshing, as reported by workers in Minnesota (3) and Canada (2). Furthermore, the machine threshed seed lots responded better to seed treatment than did hand threshed lots (Fig. 3). In general, the average increase of stand over the check for all varities and treatments, was 12 per cent for hand threshed and 120 per cent for machine threshed. This corroborates the findings of workers in Minnesota (3) and Canada (2) that injured seed gave greater response to treatment than sound seed. Spergon, Semesan, and Du Bay 1205–FF were equally effective in increasing stand of machine-threshed seed and were more effective than New Improved Ceresan.

Seed treatments were tested in the field in 1943, with Bison flax treated with the several chemical compounds. In addition to the seed from the lots used in the greenhouse trials, a seed lot threshed by machine run at medium cylinder speed was included. The results are summarized in the analysis

TABLE 5.—*Analysis of variance of emergence of flax in the field trials. Seed treatment experiment, 1943*

Source of variation	Degrees of freedom	Mean Square	F	F at 1 per cent point
Threshing treatments	2	5820	113.00**	4.88
Chemical treatments	6	199	3.86**	3.04
Treatment interaction	12	33	0.64	2.41
Error	80	52	.....	.....

** Indicates significance.

of variance presented in table 5. Treatment of seed with fungicides was highly significant. The compounds, Du Bay 1205–AK (tetramethyl thiuramdisulfide), Spergon, and New Improved Ceresan, used in this experiment were equally effective in protecting the seed. Similarly, emergence as related to threshing methods is highly significant. The average emergence per row for untreated seed threshed by hand, slow machine, and fast machine was 87, 76, and 54 plants, respectively.

DISCUSSION

The foregoing studies show that *Fusarium oxysporum* causes damping-off and seedling blight of flax in Washington. *Fusarium oxysporum* f. *lini* (*Fusarium lini*) is the only *Fusarium* heretofore reported to attack flax, and this species causes wilt as well as seedling blight. Flax wilt has not been found in Washington.

Certain organisms generally considered saprophytic, both seed- and soil-borne, also have been shown to be important enemies of successful flax culture in Washington. Such omnipresent fungi as *Penicillium* and *Alternaria* can reduce stands when seeds with injured coats are sown, but are harmless to sound seed.

Experiments in which seeds were threshed by different methods show a variation in emergence of flax. Hand-threshed seed gave good stands in contrast to machine-threshed lots. Seed threshed by machine at a medium cylinder speed gave better stands than did seed threshed at a high cylinder speed. Apparently damage is due chiefly to mechanical injury, although as reported by Moore and Christensen (3) natural injury has been found.

Flax stands were improved by the use of fungicides such as Spergon, Du Bay 1205–FF, DuBay 1205–AK, Semesan, and New Improved Ceresan. Seed with a greater amount of injury responded to a greater degree to seed treatment. It is assumed that the cracks afford soil-borne organisms ready access to the interior of the seed before the seedling has time to become established. The damage to the seed also may permit loss of moisture and subsequent death to the embryo, so that stands may be poor regardless of seed treatment. Furthermore, these injured areas would seem to be ideal places for seed-borne organisms to lodge and later cause damping-off.

SUMMARY

1. *Fusarium oxysporum* causes damping-off and seedling blight of flax in Washington.

2. Species of *Penicillium* and *Alternaria* reduce stands of flax by attacking seed with broken testas.

3. In five flax varieties studied, the percentage of cracked seed was 10–15 for Zenith and Redwing, 65–75 for Viking and Bison, and 42 for Rio.

4. Seed threshed by hand gave better stands in field and greenhouse than seed threshed by machine at medium cylinder speed. Seed threshed by machine at high cylinder speed gave the poorest stands.

5. Stands of flax in field and greenhouse were improved by the use of Spergon, Du Bay 1205–AK, Du Bay 1205–FF, and New Improved Ceresan.

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THE ZINC SALT OF 2,4,5-TRICHLOROPHENOL[1] AS A SEED FUNGICIDE

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A large number of phenols and phenol derivatives have been tested as agricultural fungicides. Although many of them have good fungicidal properties, most are highly injurious to growing plants. However, the fact that seeds are not so readily injured by chemicals suggested research on several phenols as seed protectants.

MATERIALS AND METHODS

The chemicals to be tested were prepared by mixing them with talc and grinding the mixture to fine dusts in a hammermill. The resulting dusts, measured by Gooden and Smith's[2] air permeation method, had average particle sizes ranging from 4 to 8 microns.

All cracked, discolored, and off-sized seeds were removed from seed lots to be used. Weighed amounts of the culled seed, generally 500 grams, were treated, the dust dosages being reported in the results as grams per kilogram of seed.

Mixing of seeds and dusts was accomplished by rotating in round-bottom flasks for three minutes by means of an electrically driven apparatus.

All seeds, with the exception of cotton, were grown in soil composed of 2 parts muck, 2 parts black loam, and $1\frac{1}{3}$ parts sand. It was established that this soil mixture contained pathogens such as *Rhizoctonia*, *Pythium*, and certain *Fusarium* which actively reduce seedling stands. In occasional tests macerated cultures of *Rhizoctonia solani* Kühn were added to supplement the pathogenic fungi contained in the greenhouse soil.

A higher incidence of damping-off of peas was obtained when the seeds were subjected to a short pre-cooling period which simulated cool spring temperatures so frequently favorable for optimum disease conditions in the field. The seeds were planted in 12-ounce paper pots containing greenhouse soil; the pots were uniformly watered and stored one week at 8.0° C. before they were placed in the greenhouse. Seeds not requiring cold storage were sown in flats of soil and were placed directly in the greenhouse. Cotton seed, bearing natural inoculum of the anthracnose fungus (*Colletotrichum gossypii* South., known in the perfect stage as *Glomerella gossypii* (South.) Edg.) on their surface, were sown in flats of washed sand in the greenhouse held at approximately 23° C. To supplement all tests run in the greenhouse an identical series of treated and untreated seeds was planted in clean sand in order to ascertain whether or not the chemical treatment retarded or reduced seed germination.

[1] Active ingredient in the seed fungicide Dow 9B.

[2] Gooden, Ernest L., and Charles M. Smith. Measuring average particle diameter of powders. Ind. and Eng. Chem., Anal. Ed. **12**: 479–482. 1940.

Records of seedling survival and disease-free seedlings were made after seven to fourteen days growth in the greenhouse.

RESULTS

The results obtained in a representative test with a series of chlorophenols applied as dusts at the rate of 2 grams per kilogram of seeds of Alaska pea, Henderson lima bean, and Virginia bunch peanut (machine shelled), and 4 grams per kilogram of fuzzy cotton (Delfos No. 651) are in table 1. The percentage of clean seedlings recorded is based on 200 seeds planted per treatment.

TABLE 1.—*The effect of 50 per cent chlorophenol seed dusts on seedling survival*

Chemical treatment	Percentage disease-free seedlings survived			
	Pea	Cotton[a]	Lima bean	Peanut
Phenol	63	34	48	22
2-Chlorophenol	62	3	54	16
4-Chlorophenol	54	23	43	22
2,4-Dichlorophenol	79	34	50	28
2,4,5-Trichlorophenol	86	32	70	75
2,4,5-Trichlorophenol, Na salt	86	62	74	66
2,4,6-Trichlorophenol	69	24	72	65
2,4,6-Trichlorophenol, Na salt	77	42	54	73
2,3,4,6-Tetrachlorophenol	74	58	68	69
2,3,4,6-Tetrachlorophenol, Na salt	79	59	66	64
Pentachlorophenol	41[b]	47	32[b]	67
Pentachlorophenol, Na salt	48[b]	60	30[b]	60
Untreated	71	4	35	24

[a] Seed naturally infested with *Colletotrichum gossypii*.
[b] Growth retarded.

Phenol was included in this series as a compound having known germicidal qualities. It was but slightly effective as a seed fungicide at the concentration used. This was also true of the mono and dichlorophenols. Similar results not included in these data have been obtained with other mono and dichlorophenols tested.

Most consistently effective in producing healthy seedlings were 2,4,5-trichlorophenol and its sodium salt, although the parent 2,4,5-trichlorophenol was but slightly effective on cotton seed. The 2,4,6-trichlorophenol and its sodium salt were slightly less effective than the 2,4,5-trichlorophenols, except on lima bean and on peanuts. Tetrachlorophenol and its sodium salt were generally effective but not so active fungicidally as the 2,4,5-trichlorophenols on all seed types. Pentachlorophenol and its sodium salt were no better than the mono and dichlorophenols; and, in a few instances, chemical retardation of seedling growth was observed in both the sand and soil cultures.

Those phenols having good activity were compounded with talc to give dusts of several concentrations and applied at the rate of 2 grams of the

dust composition per kilogram of Alaska pea seed. Seedling survivals based on 400 seeds planted per treatment are given in table 2.

The data indicate the superiority of the 2,4,5-trichlorophenol and its sodium salt over the other chemicals tested at all concentrations, the effectiveness increasing with the concentration. The low survival of seedlings in most of the treatments is due to the rigorousness of this experiment in which macerated cultures of *Rhizoctonia solani* supplemented those organisms contained in regular greenhouse soil. This is well illustrated by the low survival figure for the untreated seeds. Germination tests in washed sand showed chemical injury or retardation of germination to be severe where the seeds were treated with 50 per cent pentachlorophenol, slight with 50 per cent 2,3,4,6-tetrachlorophenol, and none with 50 per cent 2,4,5-trichlorophenol. Some increase in seedling survival was obtained with the

TABLE 2.—*Effect of different concentrations of chlorophenol dusts on seedling survival of Alaska pea*

Chemical treatment	Percentage of seedling survival of pea at chemical concentrations of:						
	10 per cent	20 per cent	35 per cent	50 per cent	65 per cent	80 per cent	95 per cent
2,4,5-Trichlorophenol	8	10	19	22	27	28	22
2,4,5-Trichlorophenol, Na salt	11	11	19	29	20	39	51
2,4,6-Trichlorophenol	7	6	8	8	.....	.....	.....
2,3,4,6-Trichlorophenol	0	0	0	0	.....	.....	.....
2,3,4,6-Tetrachlorophenol, Na salt	0	1	0	0	.....	.....	.....
Untreated	2	2	2	2	.....	.....	.....

concentrations higher than 50 per cent but the dust compositions were physically unsatisfactory. Normal germination of the peas was obtained in washed sand even at the three highest concentrations of 2,4,5-trichlorophenol and its sodium salt.

Continued investigations with varied dust concentrations and dosages showed that the sodium salt of 2,4,5-trichlorophenol was superior to the parent 2,4,5-trichlorophenol as a seed treatment fungicide. The sodium salt of 2,4,5-trichlorophenol was especially effective on cotton seed; its volatile action appeared to have some value as a disinfectant for the control of the seed-borne anthracnose pathogen as well as acting as a protectant against soil-inhabiting organisms active in pre-emergence killing. The efficacy of the sodium salt over the parent phenol indicated that other metal salts should be tried.

Nine metal salts of 2,4,5-trichlorophenol were prepared as dust compositions, each containing 50 per cent of the active ingredient. Alaska peas, 200 per treatment, were dusted with these materials at the rate of 2 grams per kilogram of seed and planted in soil containing damping-off organisms. Seedling survival records (Table 3) from greenhouse tests showed that the zinc, lead, copper, potassium, and barium salts were significantly better

TABLE 3.—*Effect of metal salts of 2,4,5-trichlorophenol (50 per cent active ingredient) on seedling survival of Alaska pea seeds*

Salts of 2,4,5-trichlorophenol	Percentage seedling survival
Zinc	93.5
Lead	92.5
Copper	89.5
Potassium	87.5
Barium	84.5
Sodium	80.5
Mercuric	78.0
Silver	67.5
Calcium	67.5
Untreated	74.5
Least significant difference between means (0.01 level)	3.5

than the sodium salt in the order named. All treatments, except the silver and calcium salts of 2,4,5-trichlorophenol, increased the seedling survival significantly over the checks.

In table 4 are shown representative greenhouse results of several of the best metal salt compositions compared with the sodium salt of 2,4,5-trichlorophenol applied as dusts to several types of agricultural seed, using 300 seeds per treatment.

Treatment with zinc salt of 2,4,5-trichlorophenol consistently resulted in high seedling survival for all seeds treated. Of particular note is the fact that this compound also controlled the seed-borne *Colletotrichum gossypii*. Only in the case of Detroit red beet and Virginia Savoy spinach seed treatment was the zinc salt excelled by the copper salt, although these differences may not be significant.

Seeds treated with different concentrations and dosages of zinc salt of 2,4,5-trichlorophenol were planted in the greenhouse. In table 5 are repre-

TABLE 4.—*Comparison of metal salts of 2,4,5-trichlorophenol (50 per cent active ingredient) as seed fungicides*

Salt	Percentage seedling survival					
	Dosage in grams of dust composition per kg. of seed					
	4	2	2	2	2	12
	Fuzzy cotton[a]	Lima bean	Peanut	Red beet	Spinach	Lettuce
Zinc	81	62	56	150[b]	87	85
Lead	71	60	42	123[b]	86	79
Copper	45	44	.....	168[b]	88	73
Sodium	81	34	17	99	63	75
Untreated	65	24	2	118[b]	72	77

[a] Seed naturally infested with *Colletotrichum gossypii*.

[b] Emergence figures over 100 per cent due to presence of several viable germs for each seed ball.

sentative data from cotton seed tests sown immediately after treating and 4 weeks after treating with several concentrations and dosages of zinc salt of 2,4,5-trichlorophenol. Effectiveness of the material was maintained over the 4-week period between treating and testing. From these and similar tests it was concluded that the optimum concentration of the zinc salt of 2,4,5-trichlorophenol was near the 50 per cent dust composition applied at a dosage of 3 grams per kilogram. Higher concentrations were not so easily handled and seedling emergence was reduced at higher dosages. The lowest concentration was not so effective except at the highest rate of application.

TABLE 5.—*Effect of concentration and dosage of the zinc salt of 2,4,5-trichlorophenol on seedling survival of fuzzy cotton seed*[a]

Concentration (in per cent) of Zn-trichlorophenate	Dosage, gm./kg.	Percentage disease-free cotton seedlings survived	
		Test 1[b]	Test 2[b]
100	1.5	49	65
	3.0	63	67
	6.0	47	54
	12.0	20	35
75	1.5	55	60
	3.0	52	56
	6.0	61	65
	12.0	33	44
50	1.5	49	50
	3.0	47	67
	6.0	53	66
	12.0	40	62
25	1.5	36	39
	3.0	45	43
	6.0	54	48
	12.0	51	66
Untreated	.....	8	2

[a] Seed naturally infested with *Colletrotichum gossypii*.
[b] Test 1 sown immediately after treatment.
Test 2 sown four weeks after treatment.

In table 6 are shown the results of additional greenhouse tests with dust concentrations containing 10 to 50 per cent zinc salt of 2,4,5-trichlorophenol on Alaska pea, reginned cotton seed (Mexican Big Boll), and fuzzy cotton seed (Delfos No. 651).

On pea seed, the greatest fungicidal effectiveness was obtained with the highest concentration and dosage used. There was no indication of injury from any of the treatments.

On reginned cotton seed the differences between treatments were not so great as differences among pea treatments but more consistent results were obtained with the highest concentrations. The large number of disease-free seedlings from the untreated seed indicated that this heavily reginned seed had lost much of its spore inoculum in the removal of seed fibers.

With the Delfos fuzzy seed, which carried a high spore load on the seed

fibers, the results indicated that all four concentrations of the zinc salt of 2,4,5-trichlorophenol were effective when applied at 3 grams per kilogram of seed. More exacting field studies should prove which concentration is most desirable.

TABLE 6.—*Effect of concentration and dosage of the zinc salt of 2,4,5-trichlorophenol on seedling survival*

Concentration (in per cent) of zinc salt of 2,4,5-trichlorophenol	Dosage, gm./kg.	Percentage disease-free seedlings survived		
		Alaska pea	Reginned Cotton[a]	Fuzzy cotton[a]
10	2.0	40.5	63.0	.....
	4.0	49.5	63.0	.....
	6.0	50.0	.....	.....
20	1.5	.....	.....	56.5
	2.0	52.5	58.8	.....
	3.0	.....	.....	75.0
	4.0	70.0	70.5	.....
	6.0	73.0	.....	.....
30	1.5	.....	.....	69.5
	2.0	47.0	65.5	.....
	3.0	.....	.....	77.0
	4.0	65.0	80.0	.....
	6.0	82.0	.....	.....
40	1.5	.....	.....	71.0
	2.0	63.5	71.3	.....
	3.0	.....	.....	79.0
	4.0	79.0	71.5	.....
	6.0	90.5	.....	.....
50	1.5	.....	.....	77.5
	2.0	67.5	68.0	.....
	3.0	.....	.....	77.0
	4.0	89.0	75.0	.....
	6.0	96.5	.....	.....
Untreated	.....	24.2	55.2	13.0
Least significant difference between means (0.01 level)		2.8	4.2	19.1

[a] Seed naturally infested with *Colletotrichum gossypii*.

DISCUSSION

The results given in this paper indicate that the zinc salt of 2,4,5-trichlorophenol is the most effective of the phenols tested as a fungicide for seed treatment. Preliminary tests with other phenol derivatives, not reported here, also indicated that the zinc salt of 2,4,5-trichlorophenol is the most active phenol fungicide for the greatest number of economic seeds tried. Considerable work remains to be done before optimum dilution of the active chemical and dosages of the dust can be suggested for seeds of specific plants. It is generally conceded among investigators of seed fungicides that no universal seed treatment is likely to be found and that no general recommendations can be made on the basis of results obtained on a few species.

The comparison of the metal salts of 2,4,5-trichlorophenol in table 4 pro-

vides an interesting illustration of variation in fungicidal activity of closely related compounds. For example, the sodium salt was approximately equal in activity to the zinc salt for cottonseed treatment, but it was less active on the other seed types.

SUMMARY AND CONCLUSIONS

The zinc salt of 2,4,5-trichlorophenol has been very effective as a fungicide for seed treatment. It was superior to other phenol derivatives tested.

The most effective concentration range of the active ingredient for seed treatment preparations appears to be 30 to 50 per cent of zinc salt of 2,4,5-trichlorophenol combined with an inert diluent. Dosages have been established for seeds of only a few species, but it appears that the usual dosage recommendation of 2 to 4 grams per kilogram for large seeds like cotton may be generally applicable.

In addition to reducing pre-emergence killing of seedlings by soil-inhabiting fungi, the zinc salt of 2,4,5-trichlorophenol has also been effective in reducing seedling infection by *Colletotrichum gossypii* South. when seed naturally infected by this fungus is treated before planting.

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MORPHOLOGY AND THE MODE OF TRANSMISSION OF THE RAGI SMUT

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Ragi or nachni (*Eleusine coracana* Gaertn. f.) is an important cereal grown in Mysore and in parts of the Bombay and Madras Provinces (India). In Mysore a large number of people use it for food, and extensive tracts of land are therefore under ragi cultivation. Very few diseases due to fungi are known to attack this crop but a smut due to *Melanopsichium eleusinis* (Kulkarni) Mundkur and Thirumalachar, which often does damage, was first described by Kulkarni (3) on material collected by him at Malkapur in the Kolhapur State. He named the fungus *Ustilago eleusinis*.

The mode of transmission of this smut and methods of controlling it are, however, still unknown. Kulkarni (3) states that he smeared viable spores on ragi seeds which he sowed in pots, and he claims that he obtained smutted ears in the resulting crop. Field experiments by McRae (4) did not, however, corroborate Kulkarni's findings. A more detailed investigation on the cytology of the fungus and its mode of transmission was, therefore, considered highly desirable. Pot experiments were conducted at Bangalore at the proper ragi sowing time and the results obtained are presented.[1]

DESCRIPTION OF THE SMUT

In Mysore two crops of ragi are raised in a year. The "kar" ragi is sown in February–March and harvested in June–July, the crop being cultivated in moist places following the rice crop. There is practically no smut on this crop. The second crop, known as the "hain" crop, is sown in July–August and harvested in November–December. Much damage is caused, however, to this crop by smut. The smut is first evident some time after flowering has started and is, as a rule, scattered at random in the ear (Fig. 1, 1) about five to six grains out of nearly 200 being affected. When the disease occasionally appears in an epidemic form as many as 15 grains in an ear may be attacked. The diseased grains are transformed into galls or bullate bodies, six to seven times the normal size of the grain. In the early stages of attack the affected grains are slightly greenish, and 2–3 mm. in diameter; and they project slightly beyond the glumes. As development proceeds, the attacked grains swell and reach a diameter up to 16 mm. The greenish outer tunica of the sorus gradually turns pinkish green and shows signs of rupturing at several places.

Dissection of the infected spikelet at various stages of development and a study of microtome sections of young sori reveal that the smut is strictly

[1] To Mr. M. J. Narasimhan, Director of Agriculture, Mysore, who furnished the required facilities for carrying out the experiments reported in this paper, we wish to express our deep debt of gratitude.

ovariicolous. The short style, the feathery stigma, the stamens, and the glumes are entirely unaffected (Fig. 1, 2 and 3).

CYTOLOGY OF DEVELOPMENT

Material for cytological study was collected at various stages of development in fields near Bangalore and fixed in formalin-acetic-alcohol or in Karpechenko's modification of Nawaschin's fluid. Microtome sections of 6

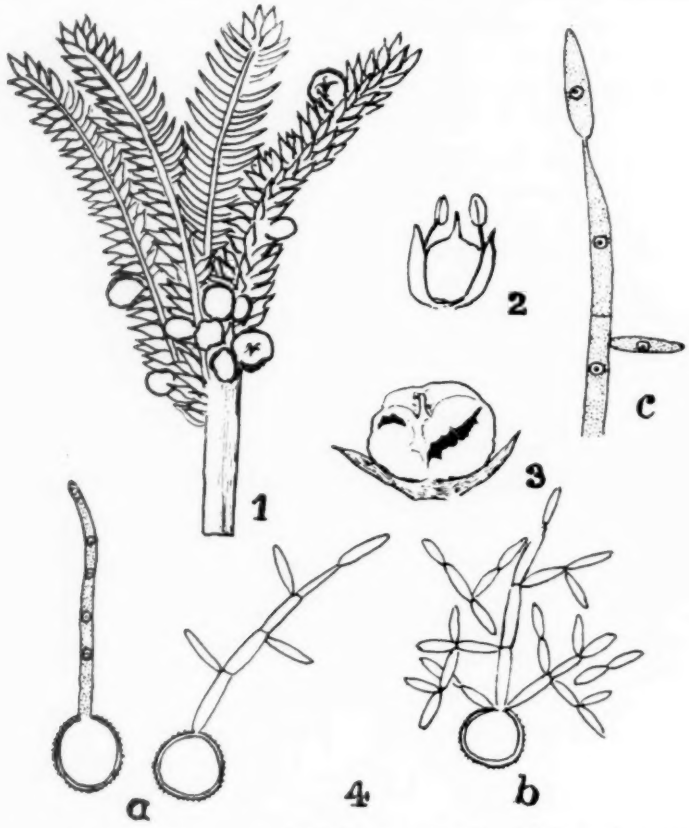

FIG. 1. The ragi smut. 1. A smutted ear of ragi. Natural size. 2. A young sorus × 2. 3. A nearly mature sorus × 3. 4. Stages in the germination of spores. a and b, × 750; c, × 1250.

to 10 μ thickness were cut and stained with Newton's iodine gentian violet or Haidenhain's iron alum haematoxylin with orange G as counter stain. The chlamydospores were germinated and then stained using the method suggested by Thirumalachar (8).

Sections through young sori show that in the initial stages of infection the ovary becomes multi-layered by the rapid multiplication of the cells. Immediately thereafter small lysigenous cavities appear at different parts of the ovary by the disintegration of the host cells. Gradually the cavities enlarge and become ovate or spherical. A thick felt of mycelium then

begins to border the cavities which become filled with a mucilaginous fluid that can be stained with Congo red or eosin B.

Chlamydospores form very soon thereafter from the mycelia bordering the cavities. Their development is thus centripetal, the oldest spores being in the center of the sorus. Young spores are somewhat polyhedral and thin-walled; mature spores are subglobose to spherical, yellowish-brown and minutely verruculose. They measure 7 to 11 μ in diameter with a mean of 9.6 μ. Due to the formation of two, three, or even four lysigenous cavities filled with these spores within a single swollen ovary, the sorus is locular, each locule being clearly and distinctly separated from the other by the host tissue (Fig. 2). Such locular sori are the distinguishing feature of the

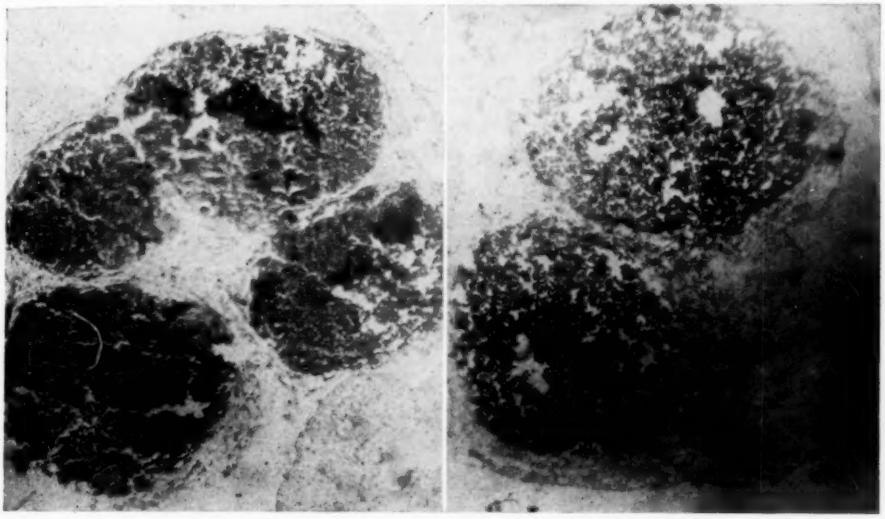

FIG. 2. Photomicrographs of the ovaries to show locular sori.

genus *Melanopsichium* Beck; and the ragi smut sori bear close resemblance to the sori of *Melanopsichium pennsylvanicum* Hirsch., one of the gall-forming smuts found on *Polygonum glabrum* in India. In this latter smut the masses of chlamydospores lie embedded in mucilage and later form tendril-like crusts because of the hardening of the mucilage. The mucilage in the sori of the ragi smut is not so copious or appreciable in quantity. All these characters led Mundkur and Thirumalachar (7) to rename the ragi fungus *Melanopsichium eleusinis*.

After the development of the chlamydospores the tissues of the ovary wall dry up and later, due to desiccation, the locular nature of the sorus becomes less conspicuous. The interior of the sorus then disintegrates and becomes a dusty mass of spores. The loose spores may be dispersed by wind but in many cases the outer rind of the host tissue covering the sorus remains persistent and the entire bullate sorus is detached from the panicle and drops to the ground. Later, as a result of the disintegration of the sorus, the spores are dispersed.

The spores germinate, without a rest period, when floated on rain water or nutrient solutions. For studying the nuclear details of germinating spores, permanent preparations were made. The promycelium first protrudes as a small papilla and then elongates into a stout germ tube. Following nuclear divisions, it becomes three-septate and bears both terminal and lateral sporidia (Fig. 1, 4). Mature sporidia are ovate to cylindric, uninucleate, thin-walled, and biguttulate. In spite of careful examination of several preparations sporidial conjugations have not been seen.

Chlamydospores germinate on potato-dextrose agar and form small white colonies within 3 or 4 days. Very little mycelium is produced in culture, but secondary sporidia are produced rapidly.

INOCULATION EXPERIMENTS

Inoculation experiments were undertaken to determine the mode of transmission of the disease.

Inoculation of seed. Two separate tests were carried out. In one set, the seed was smeared with viable chlamydospores and sown in pots. In another, the seed was smeared with sporidia from a culture and then sown. For each set of experiments ten pots were used, with five plants per pot. Experiments were carried out at Bangalore. Adequate controls were provided.

Neither in the controls nor in the pots sown with inoculated seed did the disease appear. The experiments were repeated during two more seasons, but negative results were obtained every time.

Inoculation of seedlings. In some plants that were about three to five inches in height, sporidial suspensions were injected with a delicate hypodermic needle, but no chlorotic spots or smutted grains appeared in the plants.

Floral inoculation. When it became evident that the disease is not externally seed-borne, attempts were made to inoculate the flowers at the anthesis stage using the technique suggested by Moore (5) for inoculating wheat with the loose-smut fungus. Both viable spores and sporidial suspensions obtained from the culture medium were used. As the spores do not, on germination, form promycelial infection threads but only sporidia, it was thought unlikely that the fungus would form dormant mycelium within the seed.

The sporidial suspension was well dispersed within the glumes and pervaded the entire spikelets. The inoculated ears were enclosed in paper bags and kept under close observation. None of the grains, however, became infected. The seed was preserved and sown the next year in pots to see if smut would develop in the progeny. No such smut was noticed.

It was then surmised that the maturity of the flowers at the time of inoculation had some bearing on the capacity of the sporidia to infect. Microtome sections of the spikelets with smut sori had shown that at the time the sori contained mature spores, the noninfected ovule was at the

megaspore mother-cell stage, indicating that infection must have taken place at a very early stage of development of the spikelets. Field observations had also shown that sori were present in ears that had just emerged from the sheaths.

Young ears that were still enclosed within the sheath were then selected for the inoculation experiments. The inoculum, which consisted of suspensions of sporidia or chlamydospores, was dropped into 125 such sheaths in the form of a fine jet as suggested by Eddins (2). The inoculated sheaths were often wrapped in moist cotton pads to provide moisture. Adequate controls (20 sheaths) were treated in the same manner but without spores or sporidia.

After the ears had emerged from the sheaths, the cotton pads were removed. When the ears matured, it was found that this method of inoculation was successful. Out of 125 ears that were inoculated 17 were infected, but in each ear not more than 9, and in one case 12, grains were smutted. There was no smut in ears of the control plants.

These experiments indicate that early floral infection occurs in the ragi smut and that the disease is not carried in or on the seed. Observations on the occurrence of smut sori in the ear and on the rapid production of sporidia rather than infection threads by the germinating chlamydospores support the view that the fungus is not systemic in ragi. It seems probable that ragi is infected by air-borne inoculum just as wheat is infected by the karnal bunt *Neovossia indica* (Mitra) Mundkur (6) and as bajra (*Pennisetum typhoides*) is infected by *Tolyposporium penicillariae* Bref. (1).

DISCUSSION

These investigations indicate that ragi smut is probably an air-borne disease and that it is not carried in or on the seed. This finding has made the problem of controlling ragi smut rather difficult. Treatment of seed either with fungicidal dressings or by hot water is out of the question. In efforts to discover resistant varieties there appears to be a good deal of hope, but that is a time consuming process. Removal of smutted ears, which can be readily seen in the fields, so as to prevent the sori from lodging in the ground and deep ploughing to bury the sori so as to render them innocuous are perhaps some of the other methods that merit a trial.

SUMMARY

Ragi smut due to *Melanopsichium eleusinis* (Kulkarni) Mundkur and Thirumalachar is distributed in the ragi-growing areas of Mysore, Bombay, and Madras Provinces.

Sections of very young sori which are ovariicolous indicate that the fungus forms locular sori in lysigenous cavities. The sorus converts the ovary into a large gall six to seven times the normal size of the grain. Spores are formed centripetally. They germinate readily producing primary and secondary sporidia. Conjugation of sporidia has not been observed.

Several experiments to determine if the smut is externally seed-borne gave consistently negative results. When ears that were just emerging from the sheath were inoculated with sporidial or spore suspensions, about 13 per cent of the plants became smutted. These results indicate that floral infection takes place at an early stage and the smut is probably air-borne.

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FUNGI PATHOGENIC TO BLUEBERRIES IN THE EASTERN UNITED STATES

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(Accepted for publication April 5, 1947)

Several species of blueberry (*Vaccinium*) are indigenous in the United States. The lowbush species often form dense clumps, or grow thickly over wide areas on uncultivated lands where there is little competition by larger shrubs or trees. In such situations, pathogenic fungi find conditions favorable for rapid spread. Severe leaf infections resulting in extensive preharvest defoliation sometimes occur in wild growth of the lowbush species *Vaccinium angustifolium* Ait.[3] and *V. myrtilloides* Michx. in Maine, and *V. vacillans* Torrey and *V. pallidum* Ait. in more southern regions. The highbush swamp blueberry, *V. australe* Small, grows wild in the Atlantic Seaboard States, and the rabbiteye species, *V. ashei* Reed, in southern Alabama, southern Georgia, and northern Florida. These two highbush species, while sometimes found in dense and extensive stands, occur mostly in clumps of a few bushes, or singly and scattered. Consequently the opportunity for dissemination of spores of pathogenic fungi from one bush to another, or to groups of bushes, is less favorable than if the plants were massed. Then, too, individual seedlings of the species may have a genetic resistance to pathogenic fungi.

The highbush swamp blueberry is now grown commercially in Massachusetts, Michigan, North Carolina, New Jersey, and New York. The rabbiteye form is cultivated in Alabama, Florida, Georgia, Louisiana, and Mississippi.

When blueberry varieties are grown under cultivation in solid stands of about 1,400 bushes per acre, the opportunity for spread of fungus spores from one bush to another is obviously much greater than in the case of scattered wild bushes.

With few exceptions all known parasitic fungi of the cultivated blueberry are restricted to *Vaccinium* and presumably are indigenous. Doubtless they were present in wild blueberries long before cultivated fields were established. Usually blueberries are cultivated on soils and in localities where the wild forms thrived, and it is not unusual for cultivated blueberry fields to be surrounded by forests, swamps, or cut-over lands where wild blueberry bushes form a portion of the flora, a condition very favorable for the spread of diseases from the wild to cultivated fields. A good illustration

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[2] The authors are indebted to Mr. John A. Stevenson and Miss Edith Cash for assistance and advice concerning the determination of the fungi discussed in this paper, and to Miss Cash for preparing the Latin diagnoses of the new forms described.

[3] The nomenclature of host material in this paper is that adopted by W. H. Camp in the publication entitled North American Blueberries, with Notes on Other Groups of Vacciniaceae. Brittonia **5**: 203–275. 1945.

of this type of spread of blueberry fungi has been reported by the writers (7) in the case of the blueberry cane canker caused by *Physalospora corticis* Demaree and Wilcox. This fungus is not known to be present in New Jersey, a section where the blueberry is more extensively cultivated and propagated than elsewhere. When New Jersey-grown nursery bushes are planted in North Carolina they often become infected with the canker fungus as the result of the spread of the disease from infected wild bushes near the fields. This canker fungus has been found in wild bushes growing not only in close proximity to cultivated blueberry fields but also in localities remote from blueberries under cultivation.

Lesions caused by most leaf inhabiting pathogens of blueberry are similar in size, color, and markings. As a consequence field determinations are often exceedingly difficult to make; and therefore it frequently is necessary to postpone definite identification until examination can be made by laboratory techniques.

This paper is an attempt to bring together in one publication the present knowledge concerning fungi pathogenic to blueberries, especially cultivated blueberries grown in the Atlantic Seaboard and Gulf States. Included is information about the occurrence and distribution of common and widespread forms of blueberry pathogens, some reported but little known, others reported but rarely seen, and a few heretofore not reported. With a few exceptions, the fungi herein discussed include only those that have been collected or observed by the writers. The list may not be complete, especially for forms inhabiting wild species and for those of the cultivated rabbiteye blueberry (*Vaccinium ashei*). We have a few undetermined collections of the latter.

SEPTORIA ALBOPUNCTATA CKE.

This blueberry pathogen was described by Cooke (5) in 1883 from leaves of *Vaccinium arboreum* Marsh collected in Florida and North Carolina. No later collection of the fungus or mention in literature was known to the writers previous to their collection of it from greenhouse plants at the Plant Industry Station, Beltsville, Md., in 1939. This occurrence was on plants formerly used by the late F. V. Coville in his breeding experiments at Washington, D. C. The source of the fungus found on those plants is unknown. Dr. Coville collected plants from various regions and evidently brought the fungus in on some material. The fungus has not been found in wild or cultivated plants in the vicinity of Washington, D. C. The writers have since collected the fungus on *V. australe* in U. S. Department of Agriculture experimental plots near Atkinson and Ivanhoe, N. C., and Brunswick, Ga.; also on *V. ashei* at the Coastal Plain Experiment Station, Tifton, Ga., and in commercial plantings near Crestview, Fla.

Cooke described the leaf lesions resulting from infection by *Septoria albopunctata* as small, circular, white within, and with a purple border. Usually the spots have the appearance thus described, but sometimes the

centers are tan to russet with a peripheral zone of brown, or the spot may be entirely brown.

Cooke made no mention of the attack on young shoots, though this sometimes occurs on the current year's growth. In general, lesions on shoots resemble those formed on leaves (Fig. 1, A, D), except that the shoot lesions are larger. Single lesions may be 5 to 6 mm. in diameter, circular to irregular, tan to gray, slightly sunken, and surrounded by a zone of reddish brown. A single pycnidium is usually present within the confines of the lesion.

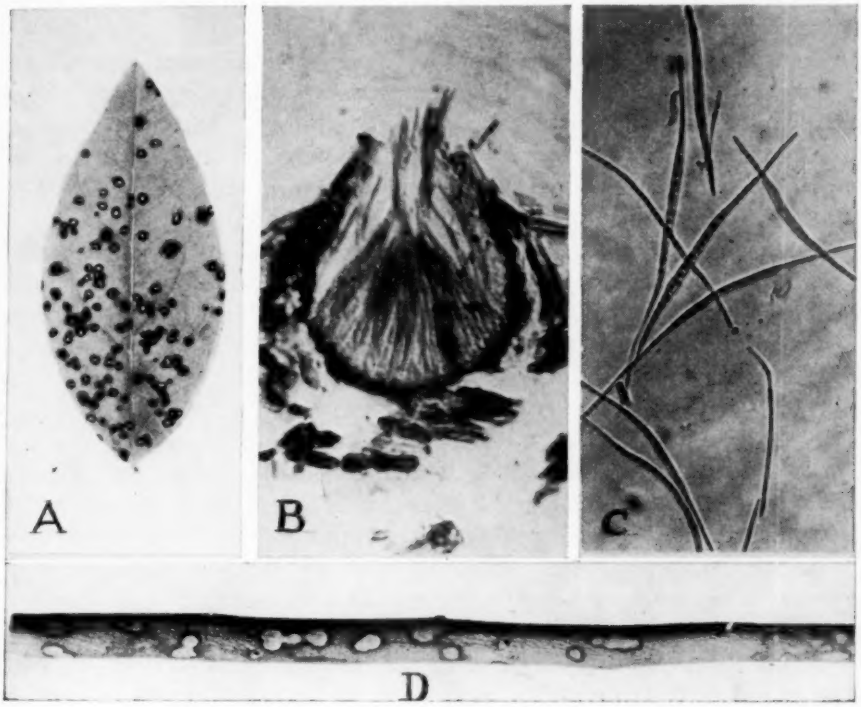

FIG. 1. *Septoria albopunctata* Cke. A. Blueberry leaf naturally infected with *S. albopunctata*. ×1. B. Cross section of a pycnidium. ×335. C. Pycnospores. ×365. D. Natural infection on blueberry shoot. ×1.

The fruiting body of *Septoria albopunctata* is a well developed pycnidium (Fig. 1, B) on the upper leaf surface. Upon maturity the ostiole breaks through the epidermis. On the leaves there is usually one pycnidium in a spot, but occasionally as many as four or five. The pycnidia are ovoid and average about 118 μ high and 90 μ wide. The walls are 4 to 6 μ thick and composed of 2 to 4 layers of cells.

The pycnospores are hyaline, straight or curved, 5- to 11-septate, filiform, obclavate to spindle-shaped, often with a long, attenuated apical segment (Fig. 1, C). When formed in host tissue they vary from 42 to 96 μ long and 3.0 to 4.8 μ wide, averaging about 70 μ by 3.6 μ. The sporophores average 12.0 μ long. When the fungus was grown on cornmeal agar the pycnospores produced were about twice the length of those measured from host tissues.

The fungus grew well on cornmeal agar and covered the sloped surface of tubes in about a week at room temperature. The aerial hyphae were greenish gray to mouse color. Pycnidia of the same type as those formed on leaves developed on the agar surface.

Pycnospores developed in cultures were used to inoculate greenhouse-grown blueberry plants. Leaves of all ages proved to be susceptible. Those that were young and growing when inoculated showed signs of infection in 12 to 18 days, but mature leaves did not show infection until the third to fourth week after inoculation. Infection took place only when the inoculum was applied to the under leaf surface.

Transmission of the disease by spraying plants with a water suspension of pycnospores was successful on all blueberry varieties and species used. These included the cultivated varieties Cabot and Rubel (*Vaccinium australe*), June and Rancocas (*V. australe* × *V. angustifolium*), Wareham (*V. corymbosum* L.), and seedling plants of *V. alto-montanum* Ashe, *V. ashei*, *V. myrsinites* Lam., *V. lamarckii* Camp, and *V. ovatum* Pursh.

Septoria albopunctata has been collected only infrequently, even though it has a wide host range. Greenhouse plants were readily and heavily infected by artificial inoculation, and nursery plants and propagating material are potential carriers of the fungus. Where it occurs the leaves are often badly spotted, which results in some defoliation.

DOTHICHIZA CAROLINIANA SP. NOV.

Dothichiza caroliniana has been found only in North Carolina. There it causes a widespread and damaging disease of cultivated varieties of the high-bush blueberry. It has not been observed on any other *Vaccinium* species. Presumably the fungus is indigenous on wild blueberries in North Carolina; however, it has not been found in the wild except near cultivated blueberry fields. The fungus is not known to attack any part of the blueberry plant except the foliage, but it sometimes causes extensive leaf spotting. In years when rains are frequent in May and June the disease becomes epiphytotic, resulting in considerable defoliation in August and September. In fact, this is the only fungus foliage-disease of blueberries of sufficient importance in North Carolina to warrant the use of a fungicidal spray.

A few spots may appear on the foliage during the latter half of May; the number gradually increases in June, July, and August. At first the spots are 2 to 3 mm. in diameter, circular, with brown centers, or sometimes light brown, rust, or gray, surrounded by a dark-brown ring. Apparently the leaf tissues build up a protective layer around the infected cells and the fungus is at first confined to a small area. Later, after midsummer, the fungus breaks through the protective zone and then invades a much larger area, causing spots several millimeters in diameter (Fig. 2, A). If the fungus spreads from only a portion of the periphery of the original spot, the result is an irregular to fan-shaped necrotic area. The brown band, or zone, surrounding the original lesion persists and the final appearance is a large

necrotic area to one side of or surrounding a smaller spot; this has suggested the use of the term "double-spot" as a common designation for the disease. This diagnostic character is useful for field identification late in the season (Fig. 2, A).

Proof of the claim that the secondary or outer necrotic area is caused by the *Dothichiza* is based upon (1) the common occurrence of the phenomenon on the leaves, (2) the occasional *Dothichiza* pycnidia found in these areas, and (3) the fact that the fungus has been cultured from tissues of the secondary invaded areas.

Pycnidia are never abundant: from 1 to 5 or 6 occur on a spot, mostly on the upper leaf surface and within the confines of the original or central infected area. The pycnidium is black and irregular, varying from globose to pear shape. The base is frequently wedged between palisade cells, as

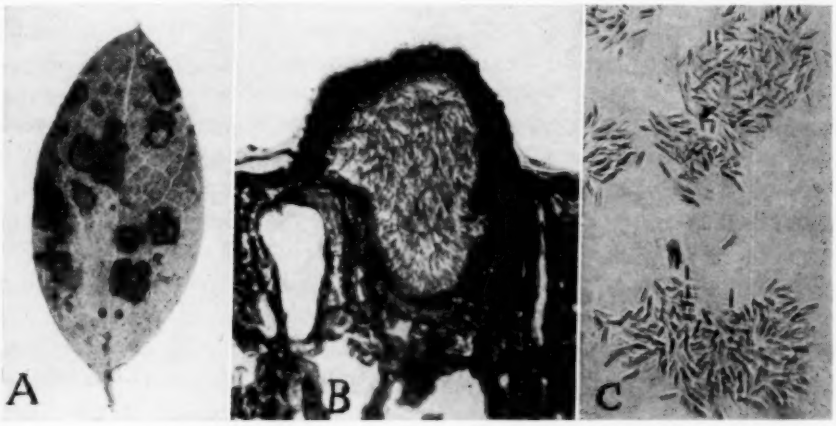

FIG. 2. *Dothichiza caroliniana* sp. nov. A. Blueberry leaf with young and old spots caused by the fungus. Note large spots with small circular primary lesion surrounded by later and more extensive invaded host tissue. ×1. B. Cross section of a pycnidium. ×335. C. A group of pycnospores. ×335.

illustrated in figure 2, B. The pycnospores are liberated through a break across the top of the pycnidium. Pycnospores have not been observed to be discharged in the form of a cirrus.

What appears to be a phase in the life cycle of the fungus is the formation of sclerotiumlike bodies in the secondary invaded area of spots. These bodies are black on the surface and have a hyaline, rubbery substance within. When they are mounted under a cover glass and pressure is exerted, they flatten out, but when the pressure is released they assume their original shape. These bodies lie wholly within the leaf tissue or sometimes partly exposed; but in one collection they were superficial. When cultured they form a growth indistinguishable from that originating from pycnospores of *Dothichiza*.

Searches have been made at numerous times in the field for an ascogenous stage of the fungus. Infected leaves have been held in the laboratory, greenhouse, and outdoors under conditions thought to be suitable for development

of a perfect stage, but none ever developed that could be associated with the *Dothichiza*.

The fungus grew slowly on agar media and became delimited before covering the slant in tube cultures. No type of fructification ever appeared in cultures, even though a variety of media was used and temperatures were varied. When autoclaved blueberry leaves were used as the substratum, sclerotial bodies formed within the tissues, but no pycnidia developed.

This Dothichiza leaf disease has been seen in almost all blueberry fields visited in North Carolina. Apparently all varieties of the highbush swamp blueberry are susceptible to infection. There is, however, some difference in degree of susceptibility. Cabot, Dixi, Pioneer, and Rancocas are the most susceptible varieties. Adams, Concord, Jersey, and Weymouth are moderately susceptible, and Grover, Harding, June, and Sam have considerable resistance.

Dothichiza caroliniana sp. nov.

Pycnidia in maculis primum 1–4 mm. diam., demum majoribus, brunneis vel griseis plerumque epiphylla, 1–5 in quaque macula, subepidermalia, erumpentia, atra, conica vel irregularia, 80–130 μ alta, 48–95 μ lata, non-ostiolata, pariete 12 μ crasso; pycnosporae hyalinae, continuae, 7.0 × 2.2 μ, utrinque obtusae, e sporophoris brevibus orientes. Hab. in foliis Vaccinii australis, North Carolina.

Spots at first 1 to 4 mm. in diameter, variable in shape, dark brown, later light brown or gray in center, often developing after midsummer a secondary necrotic area around the original spot (Fig. 2, A). Pycnidia mostly epiphyllous, usually 1 to 2 but sometimes 4 or 5 on each spot, subepidermal at first, later erumpent, slightly imbedded in host tissues, black, conical to irregular, 80–130 μ high by 48–95 μ wide (Fig. 2, B). Wall thick, about 12 μ, non-ostiolate. Pycnospores hyaline, continuous, 7.0 by 2.2 μ, obtuse at each end (Fig. 2, C), growing from base of pycnidium on short sporophores. Habitat in living leaves of *Vaccinium australe* Small in North Carolina. Type specimen, collected by J. B. Demaree at Magnolia, N. C., July 22, 1940, deposited in Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md., under No. 71489.

PHYLLOSTICTINA VACCINII SP. NOV.

A foliage disease caused by the fungus herein described as *Phyllostictina vaccinii* was first observed by the writers on cultivated bushes of *Vaccinium australe* near Beltsville, Md., in 1939. Later collections have been made on other *Vaccinium* species, both cultivated and wild, in Georgia, North Carolina, Maryland, and Mississippi. In 1942 Dr. Donald Folsom, of the Maine Agricultural Experiment Station, Orono, Me., sent the writers several cultures of fungi isolated from leaves of wild blueberries in Maine. When grown on artificial media, one of those isolates appeared to be identical with

Phyllostictina from the southern blueberry. This fungus probably is widely distributed in the eastern and southern United States, and, although unimportant as a leaf pathogen, it is potentially important as the cause of a fruit rot, since it regularly attacks fruits of one variety of *Vaccinium ashei*.

Phyllostictina vaccinii is primarily a parasite on blueberry leaves and causes small circular spots, grayish in the center and surrounded by a zone of brown host tissues (Fig. 3, A). Usually one pycnidium forms on a spot, occasionally as many as five or six. Affected bushes sometimes are partially defoliated near the end of the growing season.

Both leaves and fruits of the Black Giant, a variety of the rabbiteye blueberry (*Vaccinium ashei*), are regularly attacked. Mr. Otis Woodard estimated that 50 per cent of the berries on the Black Giant variety were infected at the Georgia Coastal Plain Experiment Station in 1942 (correspondence). On the fruits the disease appears during the preripening period as a hard, dry rot, localized in spots 5 to 6 mm. in diameter, grayish and sunken, with numerous black pycnidia in the central region (Fig. 3, B). The spots are conspicuous on the black background of ripe berries and the affected ones must be sorted out before marketing.

The fruiting bodies of the *Phyllostictina* conform to the usual concept of a pycnidium. Their size varies considerably, depending upon the substratum. On the leaves of either the rabbiteye or the highbush blueberry they average about 75 μ high and 68 μ wide, but on the fruit of the Black Giant they are much larger, averaging about 188 μ high and 165 μ wide.

The pycnospores are globose to ovoid, hyaline, granular, and average about 7.6 by 7.0 μ when formed on either leaves or fruit. Young spores (Fig. 3, E) usually possess an inconspicuous long, narrow, hyaline appendage, varying in length from 32 to 96 μ. The appendages were most clearly seen and the length easily determined when the pycnospores were first mounted in water, between a slide and cover glass; the water was then allowed to evaporate, whereupon the appendages showed distinctly. They are narrow, continuous, and frequently curved or bent near the free end.

Stained sections of the pycnidia in different stages of their development showed that the locular contents of the young fruiting bodies were composed of thin-walled pseudoparenchyma tissue (Fig. 3, C). The cells in the central region were smaller than those near the pycnidial wall. Histolysis took place progressively from the center of the pycnidium toward the periphery (Fig. 3, D). This process continued until all the original locule contents disappeared and the space was then packed with the globose, hyaline spores. Apparently no sporophores were formed during this stage of spore development, or, if formed, they became evanescent during an early period of their development. After this crop of pycnospores was discharged a second light crop of spores was formed on sporophores originating from the inner surface of the pycnidium. The sporophores were as long as the width of the spores.

This blueberry *Phyllostictina* grew well on nutrient agars and covered tube slants in a few days when held at room temperature. The agar turned black on the surface and to the depth of the penetrating mycelium. There were some mouse-colored aerial hyphae with patches of white. Prominent

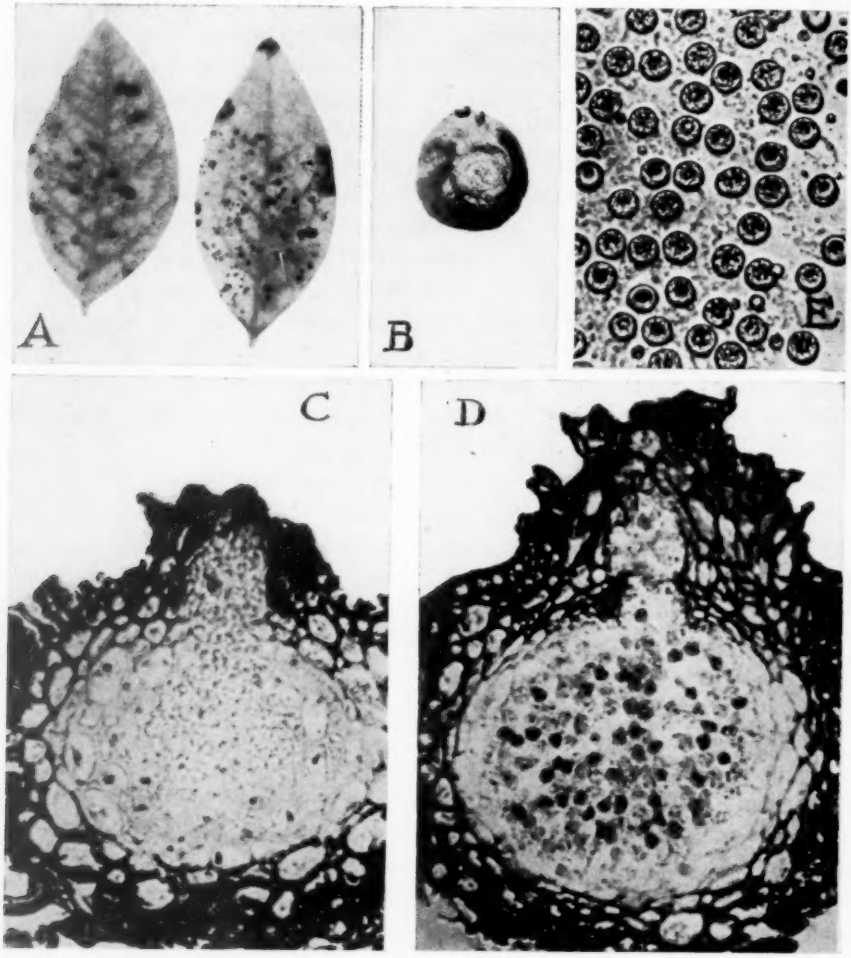

FIG. 3. *Phyllostictina vaccinii* sp. nov. A. Blueberry leaves with spots caused by *P. vaccinii*. ×1. B. Fruit of an infected rabbiteye blueberry. ×2. C. Cross section of a young pycnidium before formation of pycnospores. ×335. D. Cross section of a more mature pycnidium in process of forming pycnospores, peripheral cells of pycnidium contents still intact. ×335. E. Globose pycnospores of the fungus, some showing their delicate appendages. ×490.

stromatic bodies bearing what appeared superficially to be fruiting structures commonly formed on cornmeal agar. These structures varied in shape from spherical to tubular and we have frequently examined them with the expectation of finding them filled with pycnospores, but in all cases they were sterile. Occasionally when this fungus was grown on corn-

meal agar it formed a few slimy masses of spherical hyaline conidia on the surface of the agar, independent of any fruiting structure. The conidia were of the same size as and otherwise appeared identical with pycnospores formed in host tissues.

There is undoubtedly merit in the present tendency to break down the large and unwieldy *Phyllosticta-Phoma* form-genus when the characters of forms critically studied permit separation and assignment to some other genus.

Sydow's policy (19) of placing in the genus *Phyllostictina* those forms having fruiting bodies similar to *Phyllosticta* and filled at first with pseudoparenchymatous tissue, which later is transformed by histolysis into spores, seems to be sound. Von Hoehnel's modification (10) admits to this genus the forms having pycnidia with pseudoparenchymatous tissue subject to histolysis and producing spores having evanescent conidiophores. Shear (18) admits the presence of sporophores in a pycnidium filled with parenchymatous tissue subject to histolysis, but prefers to restrict the name *Phyllostictina* to the imperfect stage of *Guignardia*.

During the preparation of this paper some other fungi listed in literature and possibly identical with this blueberry *Phyllostictina* were examined when specimens were available, and in other cases the literature was reviewed. The following described fungi were considered: *Phoma leptidea* (Fr.) Sacc. (17) on *Vaccinium uliginosum* L., described from dead fallen leaves and having straight or curved spores 8 by 2 μ; *Phoma cymbispora* (Berk. and Curt.) Sacc. (16), growing on branches of *V. uliginosum* and having tinted boat-shaped spores 15 μ long; *Phyllosticta amicta* Ell. and Ev.[4] (9) on leaves of *Arctostaphylos sp.*, producing straw-colored spores measuring 10 by 7 μ; *Phyllosticta vaccinii* Earle (8) on *V. arboreum* reported as having spores 12 by 6 μ; *Phyllosticta cyanococci* Dearn. and House (6) on *V. corymbosum* L., reported as having spores 3 to 4 by 1.25 μ; and *Phyllosticta sparsa* Bonar (3) on leaves of *V. ovatum*, reported as having pycnidia reaching 150 μ in diameter with globular, hyaline, granular spores 12 by 9 μ, produced on simple short conidiophores up to the diameter of the spore in length.

Bonar's *Phyllosticta sparsa* resembles the eastern blueberry fungus more closely than any other fungus compared. It has pycnidia and spores larger than those of our fungus, but probably not sufficiently so to be significant. Spores of *Phyllosticta sparsa* were reported to measure 12 by 9 μ, compared to 7.6 by 7.0 μ for *Phyllostictina vaccinii*. In addition to differences in spore size, Bonar notes the presence of conidiophores and does not mention presence of spore appendages, a common character for *Phyllostictina*. Until the two forms can be compared more critically it appears best for the present to consider them as separate entities.

[4] A specimen purporting to be *P. amicta* was examined and found to be quite different from the blueberry *Phyllosticta*.

Phyllostictina vaccinii sp. nov.

Maculae in foliis plerumque 1–3 mm. diam., badiae usque griseae margine purpureo, in fructibus circulares, 6–8 mm. in diam., griseae, planae vel depressae, durae; pycnidia in foliis parca, 1–6 in quaque macula, subglobosa usque globose, epiphylla, subepidermalia, ostiolata, circa 75 μ alta, 68 μ lata, pariete crasso subcarbonaceo, in fructibus numerosa, 188 μ alta, 165 μ lata, cavitas pycnidica primum e contextu pseudoparenchymatico hyalino, dein pycnosporis ex histolysi oriundis repleta; pycnosporae subglobosae vel globosae, hyalinae, granulosae, continuae, circa 7.6 × 7.0 μ, appendicibus usque 96 μ longis, hyalinis, tenuibus praedita; conidiophori solum post liberationem conidiorum histolyticorum praesentes. Hab. in foliis Vaccinii australis, V. atrococci, et V. pallidi × atrococci, et in foliis fructibusque V. ashei, Georgia, Mississippi, Maryland, et North Carolina.

Spots on leaves mostly 1 to 3 mm. in diameter, russet to gray surrounded with purple margin. Spots on fruits circular, 6 to 8 mm. across, gray, flat or depressed, hard, with numerous black pycnidia. Pycnidia on leaves few, usually 1 to 6, oval to globose, averaging 75 μ high and 68 μ wide, on upper surface of leaf, subepidermal, ostiolate; wall thick, subcarbonaceous. On fruit, pycnidia larger, averaging 188 μ high and 165 μ wide. Pycnidial cavity first filled with hyaline pseudoparenchyma tissue, transforming by histolysis into ovoid to globose, hyaline, granular, nonseptate pycnospores averaging 7.6 by 7.0 μ and having long, hyaline, delicate appendages up to 96 μ long. Conidiophores formed only after discharge of histolysis-formed conidia.

On leaves of *Vaccinium ashei* Reade, Ellisville, Miss., Tifton, Ga., Willard, N. C., and Beltsville, Md.; *V. australe* Small, Beltsville and Salisbury, Md., Magnolia and Willard, N. C.; *V. atrococcum* (A. Gray) Heller, Buchanan, Ga.; *V. pallidum* × *atrococcum* segregate, Beltsville, Md.; and on fruits of Black Giant (*V. ashei*), Tifton, Ga. Type specimen collected by J. B. Demaree on *V. ashei*, Willard, N. C., July 30, 1942, deposited in Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md., under No. 71490.

GLOEOCERCOSPORA INCONSPICUA SP. NOV.

This fungus causes spotting of blueberry leaves and sometimes is the direct cause of premature defoliation. Collections of spotted leaves have been taken in cultivated fields of the highbush blueberry, *Vaccinium australe*, near Ivanhoe, Atkinson, and Magnolia, N. C.; on wild *V. australe* near Beltsville, Md.; and on the rabbiteye blueberry, *V. ashei*, near Ivanhoe, N. C.

The brownish lesions are circular to angular, and do not differ greatly in appearance from spots caused by *Dothichiza* before the secondary spread of the latter occurs (Fig. 4). For this reason the foliar effects caused by the two fungi are sometimes difficult to distinguish in the field.

The sporodochia of *Glococercospora* are formed more frequently on the upper than on the lower epidermis. Although there may be a dozen or more sporodochia on a spot they are usually extremely inconspicuous if the leaf surface is dry, and they often are entirely overlooked. When dry the sporodochia appear as thin, flat discs, slightly raised, and about the same color as the host tissue. When a thin film of water is spread over leaf lesions bearing the inconspicuous structures, these bodies immediately enlarge, more in height than radially, and then appear as clear, glistening, soft, spherical to conical globules. When the film of water evaporates, the

FIG. 4. Spots on blueberry leaves caused by *Glococercospora inconspicua* sp. nov.

gelatinous masses revert to the original flat disc form. This behavior is useful in identification of the disease in the field.

A satisfactory method of preparing material for microscopical study of the sporodochia has not been devised. Only a small portion of the structures remained after material was dehydrated, imbedded, sectioned, and stained in preparation for permanent mounts. Better success was obtained when hand sections were made. This method demonstrated that the sporodochia were under the cuticle or slightly imbedded in the epidermis. They measured from 40 to 90 μ wide and 36 to 60 μ high. Some were constricted at the base, as if possessing a short stipe, and widened out in a broader top. The body of the sporodochium was an undifferentiated opaque mass with some intermingling of hyphal strands. The hyphae were interpreted as sporophores remaining intact. A few conidia were usually clinging to or partly imbedded in the sporodochial cushions, and some have been seen attached to hyphae projecting slightly above the otherwise formless masses.

The conidia are hyaline, usually curved, septate, and about 35 μ long and 3 μ wide (Fig. 5). When germinating, one or more of the segments may form germ tubes.

Growth was weak and submerged on cornmeal agar and no reproductive bodies of any kind were formed. The hyphae were fine, hyaline, and septate.

A study of the early development of the sporodochia has not been made, and until that can be done one can only speculate as to the origin and morphology of the structures. The writers hold the theory that at first there is formed a palisade of simple upright conidiophores bearing conidia apically. After the conidiophores push through the cuticle and become wet by dew or rain they transform into the undifferentiated jellylike mass. The conidia are probably dispersed soon after being formed.

FIG. 5. *Glococercospora inconspicua* sp. nov. Conidia drawn by aid of a camera lucida. ×1100.

This blueberry fungus is thought to be referable to *Glococercospora*, a genus founded by Bain and Edgerton (1) in 1943 in connection with their description of the monotypic *G. sorghi*, pathogenic on *Sorghum halepense*. The two fungi are similar in several essential characters. *G. inconspicua* differs from the sorghum fungus in that its sporodochia are smaller and less conspicuous and are not confined to the stomata; the conidiophores are more completely dissolved; conidia are not formed in great abundance; growth is weak and the fungus does not sporulate in artificial culture media; and does not form sclerotiumlike bodies in artificial media or in infected host tissues.

Gloeocercospora inconspicua sp. nov.

Sporodochia in maculis fuligineis vel brunneis, interdum cinerascentibus, circularibus, 2–5 mm. in diam., epiphylla, sicca inconspicua, madida in forma globulorum gelatinosorum evidentia, subcuticularia, 45–96 μ lata, 60–85 μ alta; conidia filiformia, hyalina, curvata, septata, 35 × 3 μ; conidiophora hyalina, simplicia, madida gelatinosa. Hab. in foliis Vaccinii australis parasitica, North Carolina, Georgia, et Maryland.

Spots on leaves sooty to brown, sometimes grayish, circular to angular,

2 to 5 mm. in diameter, showing on both leaf surfaces. Sporodochia epiphyllous, inconspicuous when dry, appearing as gelatinous globules when wet, subcuticular, 45–96 μ wide and 60–85 μ high. Conidia filiform, hyaline, curved, septate, 35 by 3 μ. Conidiophores hyaline, simple, becoming gelatinous when wet. Parasitic in leaves of *Vaccinium australe* in North Carolina, Georgia, and Maryland.

Type specimen collected by J. B. Demaree at Ivanhoe, N. C., on *Vaccinium australe*, August 26, 1935, and deposited in Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland, under No. 71491.

MONILINIA VACCINII-CORYMBOSI (READE) HONEY

On April 8, 1940, the senior author, in company with N. H. Loomis of the U. S. Horticultural Field Station at Meridian, Miss., visited a small cultivated planting of the rabbiteye blueberry (*Vaccinium ashei*) near Ellisville, Miss. At that time new shoots were 2 to 6 inches long and the bushes were blooming. There was an extensive blighting of the new shoots and blossoms of many bushes, suggestive of a severe attack of the mummyberry disease, often destructive in blueberry plantings in New Jersey, Massachusetts, New York, Michigan, and Maryland, caused by the fungus *Monilinia vaccinii-corymbosi* (Reade) Honey. This Mississippi blueberry planting is a nonclonic assemblage of seedlings of the rabbiteye species; consequently there was considerable variation in susceptibility of individual bushes. While three-fourths or more of the shoots of some bushes were blighted, other bushes were moderately blighted, and still others had only a few, or no, blighted shoots or blossoms.

A search was made under the bushes for mummied berries and apothecia, but none were found. Later examination of blighted blossoms and shoots collected from the bushes April 8 did show the presence of monilioid conidia, 19–29 × 14–26 μ, connected by disjunctors.

Mr. Loomis visited this blueberry planting again during the summer, when he collected mummied berries of the current year's crop. The berries contained pseudosclerotium structures typical for the genus *Monilinia*.

Mummied berries and blighted shoots and blossoms collected from the Mississippi rabbiteye blueberry planting were sent to Edwin E. Honey,[5] an authority on the North American species of the genus *Monilinia*. He replied that the fungus was undoubtedly a *Monilinia*, but that, in the absence of the perfect stage, determination of the species could not be made. He further said, "As far as I know, this is the first report of a *Monilinia* on *Vaccinium virgatum* (*V. ashei*)."

Dr. Honey visited the Ellisville, Miss., blueberry planting the following April (1941). Fortunately, he timed his visit to coincide with a period when both apothecia and conidia were present. He concluded that the fungus

[5] Honey, Edwin E. North American Species of Monilinia. I. Occurrence, Grouping, and Life History. Amer. Jour. Bot. 23: 100–106. 1936.

was identical with *Sclerotinia vaccinii-corymbosi* Reade, which he recognizes under the name of *Monilinia vaccinii-corymbosi* (Reade) Honey.

The mummy-berry disease occurs sporadically in the northeastern United States, and sometimes causes considerable damage to the fruit of the high-bush swamp blueberry (*Vaccinium australe*). Where the fungus is present, an epidemic of the disease is contingent upon spring weather conditions favorable to the development of apothecia and dissemination of ascospores.

For the past 10 years the writers have been on the lookout for this *Monilinia* in the thriving blueberry-growing section of North Carolina, but never observed it until the spring of 1945, when a few mummied berries were seen at harvest time in one planting near Ivanhoe, N. C. This observation extends the known range of *Monilinia vaccinii-corymbosi* (Reade) Honey on *Vaccinium australe* much farther south than heretofore reported.

There is no indication except the Ellisville, Miss., observation whereby the potential seriousness of the disease in the South can be predicted.

BLUEBERRY PATHOGENS WIDELY DISTRIBUTED AND WELL KNOWN

The few blueberry pathogens critically examined and reported in the preceding sections are by no means the only ones known.

Microsphaera alni

The powdery mildew, *Microsphaera alni* DC. ex Wint., is the most widespread of all blueberry pathogens encountered. It is found at some time every summer in all blueberry fields from Maine to Florida; it causes from slight to moderate damage, and occasionally serious damage. Markin reported (11) that mildew is of common occurrence on *Vaccinium pennsylvanicum* var. *nigrum* (*V. brittonii* Porter & Beckn.) in Maine and is the cause of much early defoliation. She also reported observation of the fungus on *V. corymbosum* (*V. australe* Small), *V. canadense* (*V. myrtilloides* Michx.), and on *V. pennsylvanicum* (*V. lamarckii* Camp). The first-named species was reported to have shown very striking individual differences in susceptibility.

Bergman's (2) studies on the variation in the amount of mildew on 10 cultivated varieties in Massachusetts indicate severity of attack as follows: Pioneer, most susceptible, with Cabot and Wareham as close competitors; Concord, Jersey, and Rubel, intermediate in susceptibility; Stanley, Rancocas, Harding, and Katherine, highly resistant. From a genetic standpoint it is interesting to note that Pioneer, the most susceptible variety, and Katherine, the most resistant, are of the same parentage. Meckstroth's[6] observations in the cultivated fields in North Carolina indicate similar resistance in varieties. In his opinion Pioneer is the most susceptible variety, closely followed by Cabot. Jersey, Sam, and Stanley were moderately mildewed; Adams, Concord, and Rubel had high resistance; and a trace of

[6] Data taken from observations made by G. A. Meckstroth, Associate Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

the fungus was observed on Dixi, Harding, Grover, June, Rancocas, and Weymouth.

The powdery-mildew fungus does not attack all varieties in the same manner. On some the fungus is principally confined to the upper leaf surface, forming a white compact layer of hyphae, either localized or covering the entire surface. On other varieties the hyphae are sparse, inconspicuous, and confined to the lower leaf surface, causing superficial reddish areas where the creeping hyphae form attachments. On the upper surface of the leaves such areas are faintly yellowish spots.

Pucciniastrum myrtilli

The heteroecious rust fungus, *Pucciniastrum myrtilli* (Schum.) Arth., is about as widespread some years as the powdery-mildew fungus and likewise occurs on all *Vaccinium* species. It is most commonly found within the range of its alternate aecial host, *Tsuga sp.* This rust, however, is not confined to the regions where the hemlock is indigenous. The heaviest infection of rust on cultivated blueberry leaves has been observed by the writers in the Coastal Plain sections of North Carolina, Georgia, and Alabama—sections at least 200 miles remote from native hemlock growth. The most southern collections of *P. myrtilli* (uredia) were taken near Brunswick, Ga., on *Vaccinium australe*, May 20, 1939; Crestview, Fla., on *V. ashei*, September 25, 1939; and Boston, Ga., on *V. ashei* June 12, 1945. Abundance of the fungus in these southern sections is unusual. During the past 11 years it has been either rare or absent in North Carolina blueberry fields, except during 1938, 1939, and 1946, when the rust disease developed in epiphytotic form over a wide area in North Carolina, Florida, and Alabama and caused some premature defoliation near the end of the growing season. In 1945 the disease was so severe at Boston, Ga., on *V. ashei* that the early-formed leaves were falling and new foliage was developing prior to the middle of June. This presumably was a local outbreak. The epiphytotic outbreak in 1946 was about as severe in some blueberry fields in North Carolina as in 1938 and 1939. In 1946 this rust was observed for the first time in a blueberry field on the Eastern Shore of Maryland. An abundant infection occurred which caused considerable premature leaf fall in October. Only a light infection was observed in New Jersey that year.

The sporadic outbreaks of the rust fungus in regions remote from hemlock may be explained by assuming that there is regular overwinter survival of the uredia on southern wild species of *Vaccinium* with evergreen leaves. It is also possible that some uredia will survive in plantings of deciduous blueberries during mild winters when some leaves remain green all winter. If that is true, then the fungus could increase to an epiphytotic stage over a period of two or three years of recurrent mild winters. There is also a possibility that the occasional epiphytotics of *Pucciniastrum myrtilli* in North Carolina, south Georgia, and south Alabama originate

from spores brought in by upper air currents from some remote region where the wild hemlock grows.

Markin (11) reports that *Pucciniastrum myrtilli* is general in distribution in Maine and occurs as far as ¼-mile from hemlock trees. She has observed the rust on *Vaccinium canadense* (*V. myrtilloides* Michx.), *V. corymbosum* (*V. australe* Small), *V. uliginosum* L., and *Gaylussacia baccata* (Wangh.) K. Koch.

According to Meckstroth, cultivated varieties of the highbush blueberry are variable in their susceptibility to leaf rust. He surveyed a large planting in 1939 near Ivanhoe, N. C., composed of several varieties, all similarly exposed to infection. This examination showed that the Harding and Grover varieties were very heavily infected; Adams, Concord, Jersey, and Rubel, heavily infected; Stanley and Sam, moderately infected; Cabot, slightly infected; but no rust was found on Dixi, June, Pioneer, Rancocas, and Weymouth. In this same field in 1946, when such varieties as Grover, Sam, and Adams of the highbush blueberry were very heavily infected and were completely defoliated by early November, the varieties Owens, Myers, and Black Giant of the rabbiteye species were only slightly infected.

Although leaf rust has been reported on species of *Vaccinium* from the far western States, aecia of *Pucciniastrum myrtilli* have not been found on western hemlock species and it is suspected that the leaf rust on western *Vaccinium* either is not *P. myrtilli*, or, if it is, has some unknown alternate host.

Pucciniastrum goeppertianum

Pucciniastrum goeppertianum (Kühn) Kleb. is parasitic on certain *Ericaceae* and on species of *Abies*. It causes a disease known as witches'-broom on blueberries, which has been reported from Maine south to Pennsylvania and west to California and Washington. This disease has not been seen south of New England in cultivated blueberry fields in the East. Markin (12) says that the disease is frequent on wild blueberry plants growing within several hundred yards from woodlands and becomes serious in small areas partially surrounded by woods. In Maine the disease has been observed, according to Markin, on *Vaccinium caespitosum* Michx., *V. canadense* (*V. myrtilloides* Michx.), *V. corymbosum* (*V. australe* Small), *V. pennsylvanicum* (*V. lamarckii* Camp), *V. uliginosum* L., and *V. vitis-idaea* L. Markin states that the cultivated blueberry varieties Adams, Cabot, Harding, Pioneer, Rubel, and Sam are more susceptible to witches'-broom than are native Maine species. The varieties Katherine and Rancocas showed evidence of being resistant.

Physalospora corticus

The fungus *Physalospora corticus* Demaree & Wilcox (7) causes a cane canker of considerable economic importance in cultivated blueberries in the southeastern United States. The disease is present in most plantings of

the highbush blueberry, *Vaccinium australe,* in North Carolina and on the cultivated rabbiteye blueberry (*V. ashei*) in Alabama, Florida, and Mississippi. It has also been found on wild *V. australe* in North Carolina and in wild growths of *V. ashei* in Florida. Varieties show a decided difference in their ability to resist attacks of *P. corticus.* Of the highbush varieties, Cabot is the most susceptible and is rapidly being discarded in the South for that reason. Other observed varieties in descending order of their susceptibility are: Pioneer, Concord, June, Stanley, Jersey, Scammell, Rancocas, and Rubel. Named varieties of the rabbiteye blueberry examined for resistance are Black Giant, Hagood, Locke, Walker, Ethel, Myers, Owens, Ruby, Clara, and Scott. Cankers were found only on Locke.

BLUEBERRY PATHOGENS OF MINOR ECONOMIC IMPORTANCE OR RARELY SEEN

Twig blight, a minor disease of cultivated blueberries, is sometimes present in Massachusetts and New Jersey. This type of twig necrosis was first thought to be a form of winter injury. Later a *Phomopsis* was isolated from blighted twigs and produced the disease when the fungus was artificially inoculated in blueberry plants. The fungus is thought to enter through leaves or through the succulent shoot tips and to progress slowly downward into the scaffold branches, where it becomes perennial. It later penetrates all parts of the plant, finally causing death of the bush. The extreme symptoms are seldom obtained, since weak bushes are taken out before there is much deterioration. The signs usually seen are dead tips, from a few inches in length to 12 or 18 inches. Growers confine the incidence of the disease to a low level by pruning out the diseased shoots, cutting well below the dead portion, and burning the pruned material. Miss Wilcox (20) showed that the *Phomopsis* associated with twig blight is identical with *P. vaccinii* Shear, Stevens, and Bain, a cranberry fruit-rotting fungus. She (21) later discovered that the *Phomopsis* produced a perfect stage in culture indistinguishable from *Diaporthe vaccinii* Shear.

Brown (4) reported upon a blueberry stem and crown gall occurring on cultivated varieties in Massachusetts, Michigan, New Jersey, and Oregon, and attributed the cause to a species of *Phomopsis.* This *Phomopsis* is unlike the one causing twig blight.

Botrytis sp. is sometimes the cause of considerable loss of cultivated blueberries. The fungus attacks the blossoms, fruits, and succulent leaves during prolonged periods of foggy weather. The disease more commonly occurs in Oregon, occasionally in New England, and rarely in New Jersey.

Exobasidium vaccinii (Fckl.) Wor. has been collected on *Vaccinium* species from Maine southward to Maryland and westward to Texas and Washington. It causes a blueberry disease of minor importance but of interest because of the hypertrophy and brilliant red color of infected blossoms, fruits, and leaves.

In 1939 H. F. Bergman visited a blueberry planting on Long Island, N. Y., where severe defoliation was taking place. Fruiting bodies of a

Gloeosporium were abundant on the leaves. Specimens of diseased leaves were submitted to the writers and the fungus was determined as the imperfect stage of *Glomerella cingulata* (Stonem.) Spauld & Schrenk, the apple bitter-rot fungus. The fungus was isolated and proved to be virulent when inoculated on young leaves, succulent shoots, and green berries of potted blueberry plants (*Vaccinium australe*) in the greenhouse.

A few additional fungi pathogenic in blueberries have been collected by the writers, but they have not been critically studied. They include the following: *Ramularia effusa* Pk. on *Vaccinium vacillans* Torrey, Beltsville, Md., and on *V. lamarckii* Camp, Cherryville, Maine; *Rhytisma vaccinii* (Schw.) Fr. on *V. atrococcum* Heller, Beltsville, Md.; and *Cercosporella sp.* on *V. australe* Small, near Weymouth and New Gretna, N. J. Both collections of the last named fungus were made in 1940 and it has not been seen since. The fungus caused circular to irregular lesions 2 to 5 mm. in diameter, showing on both surfaces of green leaves. On the lower surface the spots had a slightly frosty appearance owing to a thin layer of hyaline conidia that were about 40 μ long and 3 μ wide, 1- to 3-septate, and very much curved.

Several other fungi have been reported as occurring on blueberries. Wilcox (22) listed the following: *Helminthosporium inaequale* Shear, *Melanospora destruens* Shear, *Pestalozzia guepini vaccinii* Shear, *Sphaeropsis malorum* Pk., *Dothiorella ribis* Grov. and Dugg., and *Alternaria sp.*

NAMES OF DOUBTFUL VALIDITY

Septoria difformis Cke. and Pk. was described from living leaves of *Vaccinium pennsylvanicum* collected from Lake Pleasant, N. Y., in 1875. The fungus, according to the authors (14), produced linear, straight or curved, hyaline spores in profusion, about 15 μ long and "oozing out and covering the spot with a white or glaucous bloom." The writers know of no record of collection of the fungus since. Through the courtesy of Mr. Homer D. House, New York State Botanist, Albany, a portion of the type material of *Septoria difformis* was obtained for examination. The specimen had black pycnidium-like structures on the leaf lesions, but all examined were devoid of spores. Growing between and upon the sterile structure were numerous caespitose brown conidiophores bearing conidia measuring 13–18 by 3–5 μ. These measurements fall within the range of *Ramularia vaccinii* Pk. (15). The type collection of the latter shows spots of about the same size and character as those in the specimen of *S. difformis*, but none of the black pycnidiumlike bodies are present. However, a specimen determined by H. S. Jackson as *R. vaccinii* Pk. on *Vaccinium pennsylvanicum* (Diamond Lake, Temahami Forest Reserve, Ontario, July 23, 1932) is very similar to the type collection of *S. difformis*, having spots of the same size and appearance, covered on the under side of the leaf by a dense layer of *Ramularia* spores. There were also present in this specimen, particularly around the margin of the spots, sterile black structures resembling pycnidia.

It is not clear why Cooke and Peck described this lowbush blueberry fungus as a *Septoria* when its type specimen indicates it to be a *Ramularia*.

There is one more *Septoria* reported as pathogenic on blueberry whose validity might be questioned. Miss Markin in 1931 reported (12) upon a "brown leaf spot" that was widespread and very destructive to the lowbush blueberry in some areas in the central and southeastern sections of Maine. The following year she reported (13) that the "brown leaf spot (*Septoria sp.*) nowhere caused the complete destruction of foliage that it had in 1930 and 1931." It is not known what Miss Markin was dealing with and referred to as *Septoria sp.* She gave no description of the fungus and, according to Dr. Donald Folsom, no specimens were left with the Maine Agricultural Experiment Station. The senior writer has examined wild blueberry growths in Maine but failed to find any blueberry pathogen that could be classed as a *Septoria*.

SUMMARY

This paper is an attempt to bring together in one publication the known information concerning the occurrence and distribution of fungi parasitic to blueberries (*Vaccinium spp.*) in the United States.

Particular attention has been given to fungi attacking cultivated blueberries grown in the South Atlantic and Gulf States.

Included is a summary of the rather meager literature concerning the more common and widespread forms of blueberry pathogens. Three new pathogenic fungi are described, *Dothichiza caroliniana, Phyllostictina vaccinii*, and *Gloeocercospora inconspicua*.

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RHIZOSPHAERA KALKHOFFI ASSOCIATED WITH A NEEDLE CAST OF PICEA PUNGENS

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(Accepted for publication April 21, 1947)

In the spring of 1938 a severe needle cast was observed on more than 200 trees of blue spruce (*Picea pungens* Engelm.) in a planting covering 1.5 acres at Litchfield, Connecticut. The trees were approximately 25 years old and about 20 feet in height. In the original planting the trees were spaced 6 by 6 feet, but subsequently had been thinned by removing alternate rows and, in some cases, alternate trees in the rows. Many of the lower branches on a large number of trees were completely defoliated and others bore only the young green needles of the current season's growth and those of the previous year (Fig. 1). These latter were of mature size but many of them were purplish brown and could be shaken easily from the twigs. The discolored needles bore small black superficial fruiting bodies that proved to be the pycnidia of *Rhizosphaera kalkhoffi* Bub. This fungus had not been reported previously in the United States but was known to occur on living needles of *Picea abies* (L.) Karst. in Bohemia and France (1), of *P. pungens* in Norway (2), and of *P. pungens* var. *argentea* Beiss. in Italy (1). Wilson and Waldie (7) reported it in Great Britain on species of *Picea*, and also on *Abies*, *Pinus*, and *Pseudotsuga*. Since 1938 *R. kalkhoffi* has been observed and collected in various localities in the eastern United States. It has been found only on spruce in this country, occurring on ornamental trees of *P. pungens* at Lakeville, Conn., Brooklyn, Peekskill, and Morris, N. Y., and Charlottesville, Va., and on species of *Picea* at Bar Harbor, Me., Falmouth, Mass., and Hamilton, Mass. In Canada it has been reported as abundant on *P. pungens* at Knowlton, Quebec (5). Its origin and the present extent of its distribution in North America are not known. It has never been reported on *P. pungens* in its native range.

The fungus seems to affect first the needles of the lowest branches and gradually progresses up the tree. The earliest indication of the disease, usually occurring late in the summer, is a yellow mottling of the mature needles of the current season's growth. In some of the discolored areas small tufts of brown thick-walled hyphae are found protruding from the stomata. Occasionally a few scattered needles on the newly developed twigs gradually become purplish brown and, in the spring, small black globoid pycnidia become evident above the stomata on all surfaces of the needles. The small white waxy mass that normally fills the stomatal opening adheres to the top of the pycnidium until it is mature. On severely diseased trees the infected needles usually fall during their second summer, leaving only the needles of the current season's growth.

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The pycnidia and spores on the infected needles corresponded with those of the type specimen[2] of Bubák's *Rhizosphaera kalkhoffi* and with his description of the fungus (1). The development of a pycnidium begins with the formation of a closely interwoven mass of hyaline and brown hyphae in the intercellular space below a stoma. A very few brown hyphae grow up through the stomatal cavity and branch out as they emerge above the stoma. By the repeated division of the cells of these hyphae the pycnidium is

FIG. 1. Blue spruce tree affected with needle cast. Photograph by H. G. Eno.

formed. The brown hyphae in the stomatal cavity between the guard cells develop into a well-defined stalk, characteristic of the genus (Fig. 2, A). The pycnidial wall is composed of a single layer of brown angular cells, enclosing the pseudoparenchymatous tissue from which the spore-bearing cells develop. The spores are produced histogenously by budding from any of the cells lining the pycnidial cavity. The mature pycnidia rupture irregularly and widely, exposing the spores, which are ovoid, unicellular, hyaline, with a slight brownish tinge in the wall at maturity, and measure 7–10 × 3–5 μ. No ascogenous stage has been observed.

[2] The writer is indebted to Dr. G. M. Reed, formerly Curator of Plant Pathology at the Brooklyn Botanic Garden, Brooklyn, N. Y., for the loan of Bubák's type specimen on file in that herbarium.

Before germination, the spores of *Rhizosphaera kalkhoffi* increase to about twice their normal size and, within 24 hours, send out germ tubes from both ends. The portion of the germ tube immediately adjacent to the spore soon becomes septate and a succession of short cells results. These cells branch profusely, forming a dense cluster of small-celled hyaline hyphae around the spore, which soon loses its identity in the rapidly growing hyphae

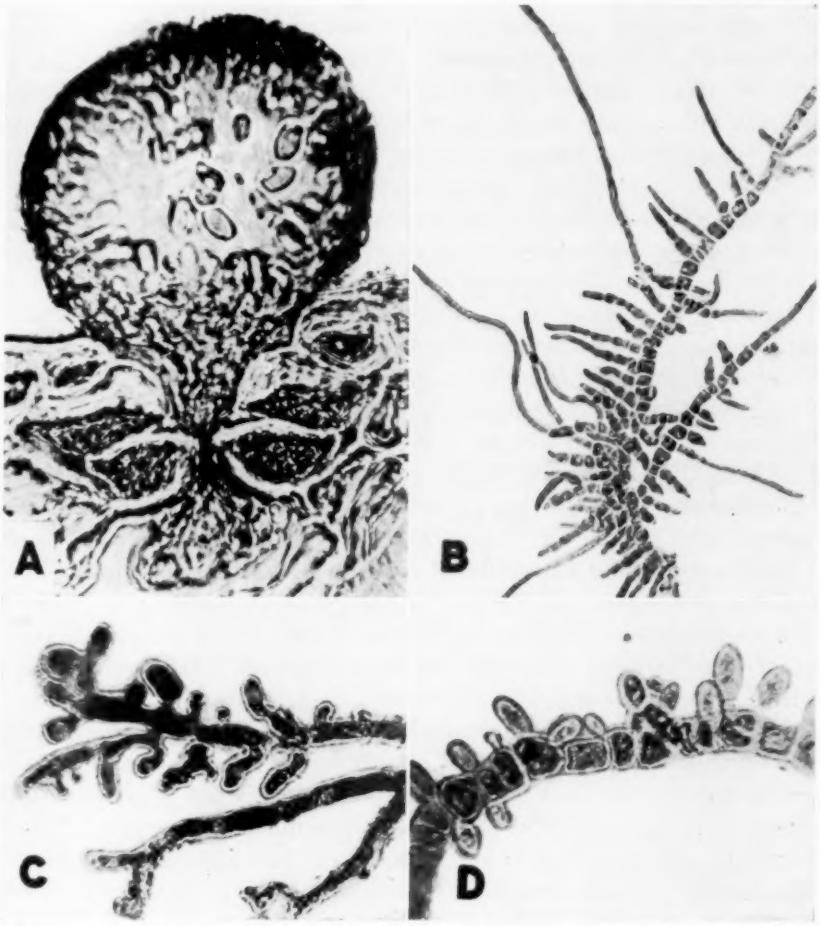

FIG. 2. A. Section through pycnidium of *Rhizosphaera kalkhoffi*, showing stalk in stomatal cavity. ×680. B. Germinating spore—2-day growth. ×165. C. Branched tip of hypha—10-day growth. ×165. D. Spores budding from hypha in culture. ×680. Photomicrographs by H. G. Eno.

(Fig. 2, B). The terminal cells of the hyphae are long, slightly enlarged at the tip, and filled with dense cytoplasm. After 10 days on Leonian's medium (3) in Petri-dish cultures some of the tips of the hyphae from a single spore become bulbous and thick-walled with a profusion of short branches (Fig. 2, C). Most of the hyphae except the growing tips eventually have thick walls, more or less knobby or uneven in thickness, and

change gradually from hyaline to brown. The hyphae resemble rather closely those found in the leaf tissue, particularly when the pycnidia are just beginning to be formed.

When grown on Bacto malt extract medium (4, p. 455) the color of the older mycelium in mass is at first "raw umber,"[3] and gradually darkens until it becomes black. Aerial hyphae are usually absent, both on the malt extract medium and on Leonian's medium. The advancing hyphae of a colony remain closely appressed to the surface of the medium and soon begin to produce an abundance of spores, which bud off in succession from any point along the hyphal cells (Fig. 2, D), and occasionally from the tips of short unicellular or long multicellular branches. The spores are loosely held together in gelatinous masses along the hyphae, giving a butyrous appearance to the surface of the culture. They are ovoid, unicellular, hyaline at first, with a brownish tinge in the wall at maturity, and are slightly wider than the pycnospores in nature, measuring 7–9.8 × 4.4–6.6 μ (25 spores). In the older portions of the hyphae several adjacent sporulating cells divide and increase in width, with a thickening of the walls. The presence of these dividing cells and their gelatinous masses of spores in series at irregular intervals along a hypha give it a zigzag appearance. The cells gradually become greenish brown and finally dark brown and thick-walled, when spore production ceases. Cell division may continue until a large, more or less globoid mass of cells is formed. In some cases small hyaline cells are produced from the outer portion of these masses by division and are easily separated from the brown cells. They resemble the spores so closely that they cannot readily be distinguished from them in mass. No pycnidia have been found in culture.

No inoculations have been undertaken by any investigator to determine definitely the pathogenicity of *Rhizosphaera kalkhoffi*, its host range, and the particular conditions under which infection takes place. The presence of intercellular hyphae in the mesophyll of the leaf when the needles begin to show discoloration and the small tufts of hyphae in the stomata give evidence of the relation of the fungus to the disease. Infection seems to be confined to the needles. The uninjured terminal buds continue to develop new growth each year until the twigs become so weakened by premature defoliation that the new growth is stunted and the young needles are readily susceptible to infection. This results in a very slow progress of the disease throughout the tree. Even on severely diseased trees the growth of the unaffected branches in the upper portions of the trees continues at a normal rate. The significant damage is largely confined to the lowest branches and therefore decreases the value of affected trees for ornamental purposes. Wilson and Waldie (7), in Great Britain, reported the death of 14- to 20-year-old trees of *Picea pungens* resulting from defoliation by *R. kalkhoffi*, but no case of the death of entire trees from the needle cast are known in the United States.

[3] The descriptive color term is that given in Ridgway (6).

Satisfactory control of the disease on ornamental trees grown alone or in small groups may be obtained by spraying. Selected trees in the planting at Litchfield, Conn., were sprayed with 4–4–50 Bordeaux mixture, to which casein was added as a spreader. Three applications were made at intervals of two weeks, beginning the latter part of June when the needles of the new growth had developed. During the two seasons when the spraying experiments were carried on, the progress of the disease was arrested by the spray on those trees where infection had caused defoliation of some of the lowest branches. Neighboring trees that showed only slight infection before spraying were effectively protected from further spread of the disease.

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FUNGI ASSOCIATED WITH RUNNER PEANUT SEEDS AND THEIR RELATION TO CONCEALED DAMAGE[1]

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INTRODUCTION

An internal discoloration and decomposition of runner peanut seeds that cannot be detected in unbroken seeds (Fig. 1), has become of considerable concern to peanut processors. This damage, generally known as "concealed damage" or "hidden damage," has necessitated the development of specific sampling methods by peanut brokers. At present many lots of peanuts offered for sale are graded down or rejected because of concealed damage.

The hyphal mats, which are usually found between the cotyledons of seeds with concealed damage (Fig. 1, B and C), indicate that the condition is a result of the action of filamentous fungi. As a first step in a general study of concealed damage, the fungi associated with runner peanuts in all conditions are being identified, and their connection with concealed damage is then being checked.

Investigations to date have revealed some definite relationships between species of fungi and damage. It seems advisable, therefore, to present an introductory report at this time.

REVIEW OF LITERATURE

All references to fungi associated with peanut fruits or to concealed damage to date have been in the form of short notes. Evans and Poole (1) in a report on fungi isolated from lesions on the seed coats and shells listed *Fusarium spp.*, *Rhizoctonia solani* Kühn., *Rhizopus sp.*, *Botrytis sp.*, *Pythium sp.*, *Sclerotium bataticola* Taub., and *S. rolfsii* Sacc. of the more common fungi, along with *Cephalothecium sp.*, *Trichoderma sp.*, *Penicillium sp.*, and *Aspergillus sp.* Prince (3) listed *Fusarium spp.* and *Alternaria spp.* as the predominant organisms associated with various bunch-peanut seeds, with *S. bataticola*, *S. rolfsii*, *Diplodia natalensis* Evans, *Trichoderma sp.*, and *Penicillium spp.* of moderate frequency. *R. solani*, *Rhizopus spp.*, and other fungi were isolated infrequently. The fungi noted by Prince were isolated from seeds, some of which appeared to have concealed damage. Concealed damage, however, is regarded as of little consequence in Spanish type peanuts (2).

Apparently the first published note on concealed damage was that of

[1] Paper No. 166, Journal Series, Georgia Agricultural Experiment Station, Experiment, Georgia.

[2] This investigation of concealed damage, begun by the junior author, was continued by the senior author after October, 1945.

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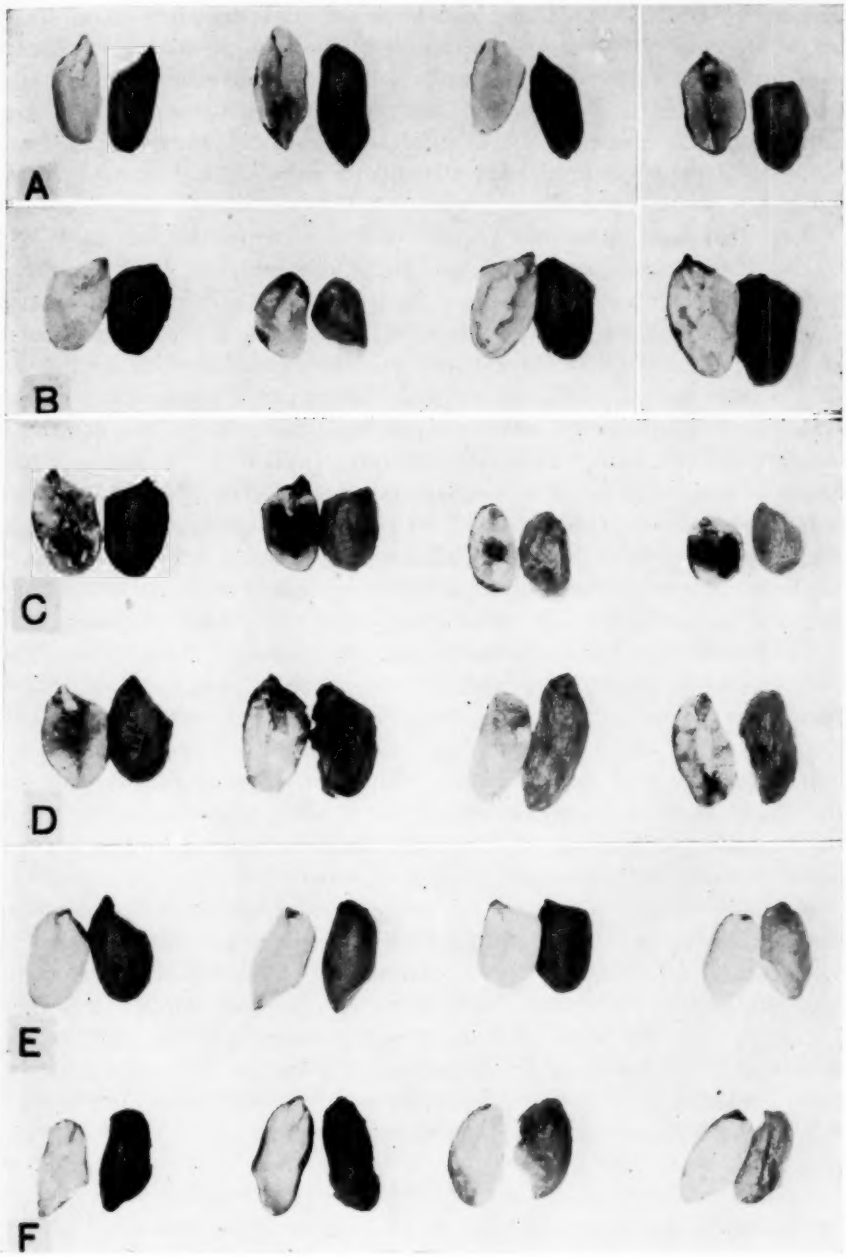

FIG. 1. Concealed-damage seeds of Southeastern runner peanuts contrasted with sound seeds. A. Prominent yellow discoloration of cotyledons, no fungus mat. B. Prominent mats of fungus hyphae between cotyledons. C. Sclerotia of *Sclerotium bataticola* in intercotyledonary space. D. Late stage of concealed damage. Discoloration of cotyledons faintly discernible through undamaged seed coat. E. Sound seeds with undamaged seed coats. F. Seeds with discolored seed coats, cotyledons sound.

Rankin (4) in 1937 who found confectioners rejecting from 35 to 40 per cent of Georgia's 1936 runner peanut crop because of concealed damage. Some lots were rejected by oil mills. After examination and isolations Rankin concluded that concealed damage was caused by several soil fungi, particularly *Sclerotium bataticola* and *Rhizoctonia spp.*, that had penetrated the shells of the nuts when harvested plants were stacked in a wet, green condition.

Concealed damage has only recently received further attention. In 1944 Higgins (2) listed common molds and fungi such as *Rhizoctonia sp.*, *Sclerotium bataticola*, *Penicillium sp.*, and *Rhizopus sp.* as isolates from concealed damage seeds. Subsequently Wilson (5) isolated a *Diplodia sp.* from 65 per cent of concealed-damage seeds examined. Ninety-five per cent of Wilson's isolates were *Diplodia sp.*, with *S. bataticola*, *Fusarium spp.*, *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.*, and bacteria making up the remainder. On this basis Wilson considered the *Diplodia sp.* to be the primary organism associated with concealed damage. Later Wilson (6) found *Diplodia theobromae* (Pat.) Nowell the predominant organism in concealed-damage seeds, while *S. bataticola*, *Rhizoctonia sp.*, and miscellaneous molds and bacteria were associated with visibly damaged seeds. Thus, Wilson indicated that a *Diplodia sp.* alone is responsible for concealed damage.

In a recent publication Wilson (7) reported isolating the same molds and soil-borne fungi from young peanuts and regarded this as proof that the young peanut fruit is invaded by fungi soon after it penetrates the soil. In this same report Wilson stated that more than 80 per cent of the isolates from seeds in early stages of concealed damage are *Diplodia theobromae* with other fungi such as *Fusarium spp.* being isolated more frequently as concealed damage progresses. He regarded these fungi from seed in advanced stages of concealed damage as secondary invaders.

Evidence to date indicates that concealed damage is not found to any considerable extent in freshly dug peanuts. Some unpublished results from this laboratory showed no lots of peanuts with concealed damage at time of digging in 1945 and one of sixteen lots with concealed damage (2 per cent) at digging time in 1946. Results from Alabama (8) showed concealed damage in 13 of approximately 100 freshly dug lots in 1945. One lot showed 12 per cent concealed damage, one 3 per cent, and one 2 per cent, while the remaining ten lots all contained less than 1 per cent.

PROCEDURE

The nature and development of the peanut fruit suggests that many soil inhabiting fungi may be associated with the mature pod; thus, concealed damage may be assumed to develop as a result of the action of fungi normally associated with the fruit. This hypothesis has been tested by a series of laboratory studies leading up to flask inoculations as a final check on the relationship of various fungi to concealed damage.

Since this was not a detailed mycological investigation, most of the fungi

encountered were identified to genus only. The species of some fungi was obvious. When it appeared that more than one species of a genus had been isolated, the apparent species were labeled "isolate No. 1," "isolate No. 2," etc. Since the main concern was with fungi present in or on seeds, no isolations were made from shells.

The Southeastern runner variety (North Carolina runner) was used throughout.

1. *Identification of fungi associated with sound seeds.* In this study fruits were used from a combination of two lots of peanuts sun-dried immediately after plowing-out. Examination of 2,000 seeds from each lot revealed no concealed damage or visible damage of a fungus nature. Isolation of fungi was started approximately one month after harvest.

Approximately 200 fruits were surface sterilized by soaking them for two minutes in acidified 1 : 500 mercuric chloride solution and then washing them in sterile water for 5 minutes. The seeds were removed and split under aseptic conditions, and all seeds having exterior or interior discoloration or in which there was any possibility of outside contamination were discarded. One-half of each remaining seed was plated on sterile, acidified tap-water agar. Two hundred thirty-two half-seeds were plated.

2. *Identification of fungi associated with concealed-damage seeds.* Peanuts were obtained from 25 stacks in which nuts with concealed damage had been found. Approximately 150 apparently sound seeds from each stack were surface sterilized and split aseptically. Both cotyledons of 556 seeds with concealed damage were plated. Fungi grew rapidly from every seed plated.

3. *Identification of fungi associated with visibly damaged seeds.* Visibly damaged seeds were selected from ten seed-lots of various origins. Seeds in advanced stages of decay were discarded, and the remainder were surface sterilized, split aseptically, and plated. Fungi were isolated from 421 seeds.

4. *Inoculation tests on the relationship of various fungi to concealed damage.* In the procedure described, 17 fungi were isolated (Table 1). Each was used to inoculate peanut fruits. Isolates of *Sclerotium rolfsii* and *Pythium sp.* from peanut pegs (gynophores) were used also.

Fruits containing at least 50 seeds were surface sterilized and transferred aseptically to a flask containing the desired fungus growing on autoclaved winter pea seeds. The flasks were incubated at 25° C. for 4 to 40 days. At the end of each period fruits were removed, air dried, and the seeds removed. All seeds with visible damage were discarded. The remaining seeds were surface sterilized, split aseptically, and those with concealed damage were plated.

Two noninoculated lots were run as controls; one with surface sterilized fruits, one with fruits not sterilized.

5. *Determination of changes in fungus flora of seeds under conditions conducive to growth of fungi.* Surface-sterilized nuts from the sound lot were transferred aseptically to flasks containing autoclaved peanut cotyle-

dons. Seventy-five nuts were put in each flask. The substratum was intended as a source of both moisture and supplementary food.

These flasks were incubated at 25° C. for varying periods, after which the pods were removed and dried. The seeds were removed, surface sterilized, and split aseptically, and one cotyledon from each seed was plated. Occurrence of concealed damage and the various stages of visible damage was noted. The number of seeds thus plated always exceeded one hundred. Time intervals were 4, 7, 10, 20, 30, 35, and 40 days. As a control 20 fruits per time interval were incubated in individual test tubes on sterile, moist filter paper.

TABLE 1.—*Fungi isolated from sound and damaged runner peanut seeds*

Fungus	Percentage of seeds in indicated condition from which fungus was isolated		
	Sound—no damage	With concealed damage	With visible damage
Diplodia sp.	1.7	89.6	5.6
Sclerotium bataticola	1.0	3.5	14.9
Rhizopus sp.	11.2	1.6	21.0
Aspergillus spp. (2 isolates)	9.9	.....	2.2
Penicillium spp. (2 isolates)	1.3	0.5	.....
Fusarium spp. (6 isolates)	1.3	0.7	5.0
Mucor sp.	0.9	.....	1.0
Haphlographium sp.	.....	.....	1.0
Rhizoctonia solani	.....	0.9	.....
Neocosmospora vasinfectum	[a]	.....	.....
Sterile fungus	0.4	.....	.....
Diplodia sp. in mixture	.....	1.6	.....
S. bataticola in mixture	.....	0.7	.....
Miscellaneous mixtures	.....	0.7	.....
Bacteria	.....	0.2	49.3
Nothing isolated	72.3	.....	.....

[a] *Neocosmospora vasinfectum* (Atk.) Smith was isolated from seeds after 7 days' incubation.

RESULTS

Table 1 gives the fungi isolated from seeds and the frequencies of these isolations. The results of inoculations to determine the relationship of eight of the species of fungi tested to concealed damage are presented in condensed form in table 2. The results for the other fungi tested were about the same as those for *Mucor sp.* and *Rhizopus sp.* with only *Diplodia sp.* and/or *Sclerotium bataticola* isolated from concealed-damage seeds.

With increasing length of incubation period for noninoculated fruits there were notable changes in the frequencies with which various fungi were isolated. The fungi that developed in peanuts incubated individually in test tubes were not significantly different from those that developed when pods were incubated together in flasks. Since this is regarded as indicating no appreciable spread of fungi from fruit to fruit, the results were combined and are given in table 3.

DISCUSSION

Fungi isolated from sound seeds removed aseptically from surface-sterilized pods were species usually encountered as saprophytes on dead plant tissue (*Sclerotium bataticola, Diplodia sp., Fusarium spp., Rhizopus sp., Aspergillus spp.*, etc.). Thus, living mycelium or spores of these fungi were present on the surface of the seeds, between the seeds and the shell, or within

TABLE 2.—*Results of inoculations of runner peanut fruits in flask cultures*

Inoculum and incubation period	Results			
	Concealed damage	Visible damage	Isolations from seeds with concealed damage	
	per cent	*per cent*	*Fungus*	*per cent*
Control: surface sterilized fruits				
7 days' incubation	0	2	.....	.....
10 do	16	8	*S. bataticola*	73
			Diplodia sp.	27
18 do	9	42	*S. bataticola*	64
			Diplodia sp.	36
Control: nonsterile fruits				
7 days' incubation	0	0	.....	.....
10 do	3	4	*S. bataticola*	66
			Diplodia sp.	33
18 do	4	26	*S. bataticola*	75
			Diplodia sp.	25
Diplodia sp.				
7 days' incubation	12	4	*Diplodia sp.*	100
10 do	44	14	do	100
18 do	20	35	do	100
Sclerotium bataticola				
7 days' incubation	11	6	*S. bataticola*	100
10 do	28	7	do	100
18 do	28	32	do	100
Fusarium sp. No. 5				
18 days' incubation	21	49	*S. bataticola*	90
			Fusarium sp. No. 5	10
Fusarium sp. No. 6				
18 days' incubation	8	53	*Fusarium sp.* No. 6	50
			Mucor sp.	50
Neocosmospora vasinfectum				
18 days' incubation	25	2	*S. bataticola*	66
			Rhizopus sp.	33
Penicillium sp. No. 1				
18 days' incubation	14	26	*Diplodia sp.*	80
			Rhizopus sp.	20
Mucor sp.				
18 days' incubation	3	11	*S. bataticola*	100
Rhizopus sp.				
18 days' incubation	16	7	*Diplodia sp.*	75
			S. bataticola	25

the seeds. Fungi, other than those isolated from sound seeds, must also be considered as members of the fungus flora of peanut fruits in curing stacks. For example, *Neocosmospora vasinfectum* and additional *Fusarium spp.* developed when sound fruits from the same lot were incubated; and one parasitic form, *Rhizoctonia solani*, was isolated from peanut fruits taken directly from stacks.

Influencing factors are too numerous to expect uniformity between localities or fields in regard to the fungus flora of peanut fruits. It should be anticipated, however, that some fungi will be found consistently because of their universal distribution in the soils of the peanut belt. A comparison of fungi isolated in this and other studies (1, 2, 3, 5, 6, 7) places *Rhizopus sp.*, *Sclerotium bataticola*, *Diplodia sp.*, and the species of *Fusarium* in this classification. Occasionally one of these anticipated species may not appear in a series of isolations, probably because of the action of such factors as antagonisms between soil fungi. Since Wilson (7) has found most of the fungi reported from mature peanut fruits also present in young peanuts soon after they penetrate the soil, it is apparent that these fungi are actively associated with the developing fruit over a considerable period of time.

TABLE 3.—*Changes in fungus flora of uninoculated peanut fruits under conditions conducive to growth of fungi, and the corresponding development of concealed and visible damage*

Fungus	Percentage of seeds from which fungus was isolated for various incubation periods							
	0 days	4 days	7 days	10 days	20 days	30 days	35 days	40 days
Nothing isolated	72.0	73.5	64.0	56.0	27.0	4.5	2.0	0.0
Rhizopus sp.	11.0	21.3	19.5	16.0	16.5	2.0	1.5	0.0
Diplodia sp.	1.8	2.4	4.0	7.0	10.5	2.3	1.0	0.0
Sclerotium bataticola	1.0	1.0	10.3	19.5	22.4	2.5	2.0	0.0
Fusarium spp.	1.3	0.6	0.4	1.0	1.9	2.5	2.0	3.5
Other filamentous fungi	12.9	1.2	1.8	0.5	9.1	20.2	26.0	32.0
Bacteria	0.0	0.0	0.0	0.0	12.7	66.0	65.5	64.5
	/— No visible damage —/—— Visible damage ——/							
	/—— Concealed damage ——/						/—— Decay ——/	

Isolation of the fungi listed in table 1 shows that there has been a general invasion of the pod by fungi. Study of concealed-damage seeds from field lots showed that the fungi concerned entered the intercotyledonary space, undoubtedly through the placenta, and spread over the surface of the cotyledons. Eventually there is considerable penetration of the cotyledons with accompanying discoloration and decomposition. On the other hand, almost all visible damage that can be attributed to the activity of fungi evidently results from the growth of fungi in the space between the shell and the seed. A good portion of this visible damage is damage to the seed coat only and is not accompanied by discoloration or decomposition of the cotyledons.

Once these fungi had invaded the shell, either in the soil or in the stack, there were, of course, two means of further development: one through the placenta into the intercotyledonary space, and the other from the shell into the space between the shell and the seed. The development of concealed damage in surface sterilized, noninoculated nuts (Table 2) shows that there can be an invasion of the intercotyledonary space by fungi commonly present and developing when conditions are favorable.

As far as species are concerned the fungi isolated from concealed-damage seeds were almost identical with those isolated from visibly damaged seeds and from sound seeds (Table 1). This indicates that much of the damage in each of the two types of damage is not only the result of the action of the same fungi but is also the result of the action of fungi normally associated with the mature pods. In addition, the results from noninoculated incubated pods (Table 3) show that concealed damage may eventually become visible damage. Thus, as previously indicated by Wilson (6), concealed damage is one of the early stages in decay and under proper conditions develops into visible damage. This does not mean, of course, that all decay or other fungus-produced visible damage results from preceding concealed damage. It is probable that only a very small percentage of the evident decay or other visible damage found in mill-run runner peanuts had its beginning in concealed damage.

It is evident from table 2 that numerous competitions and antagonisms develop when peanut fruits with their natural fungus flora are inoculated with specific fungi. Certainly *Diplodia sp.* and *Sclerotium bataticola* were the more vigorous in these competitions since they developed in almost all flasks regardless of the inoculum used. Moreover, when *S. bataticola* was used as inoculum, no other fungus was isolated from any of the damaged seeds. Results given in table 2 indicate that *Rhizopus sp.* and *Mucor sp.* and certain isolates of *Fusarium sp.* are sometimes capable of considerable development in competition with other fungi. As suggested by the results from flask inoculations with *Fusarium sp.* (isolate No. 6), *Neocosmospora vasinfectum*, and *Penicillium sp.* (isolate No. 1) other antagonisms might result in reduced activity by either *Diplodia sp.* or *S. bataticola*. Competitions and antagonisms similar to those developing in the inoculated flasks undoubtedly are active in field soils and in stacks of curing peanuts. This would explain the preponderance of isolations of *Diplodia sp.* and *S. bataticola* from concealed-damage seeds from the field.

As demonstrated herein, conclusive tests to determine which fungi cause concealed damage are difficult because of the presence of various fungi in sound fruits. This difficulty is emphasized in the inconsistent results given in table 2 for most fungi used. These results, however, may be taken as demonstrating that both *Diplodia sp.* and *Sclerotium bataticola* may produce concealed damage. More concealed-damage seeds developed in sterilized fruits inoculated with these fungi than developed in any of the other lots, and the fungus used in inoculation was reisolated from all of these seeds. The connection of these fungi with concealed damage is further emphasized in table 3 where the development of concealed damage in noninoculated fruits coincided with the period of major development of *Diplodia sp.* and *S. bataticola*.

For the 1945 season *Diplodia sp.* (Table 1) was by far the most important cause of concealed damage in the fields sampled. The action of *Sclerotium bataticola* in the inoculation tests show that in other seasons, in

other fields, or under varied conditions *S. bataticola* might equal or exceed *Diplodia sp.* in producing concealed damage. It should be noted that both Rankin's (4) and Higgin's (2) lists of fungi isolated from concealed-damage seeds included *S. bataticola* but did not include *Diplodia sp.*

There is no reason, however, for excluding other fungi from consideration as possible agents in the production of concealed damage. These inoculation tests show only that under the conditions in the flasks *Diplodia sp.* and *Sclerotium bataticola* are capable of producing concealed damage and also are capable of outgrowing or suppressing most of the other fungi that were associated with the peanut fruits. Since concealed damage results from saprophytic action, and since several fungi are associated with peanut fruits, it is evident that the action of fungi other than *Diplodia sp.* and *S. bataticola* may result in concealed damage. There were several concealed-damage seeds from which fungi other than *Diplodia sp.* and *S. bataticola* were the only organisms isolated (Table 1). *Diplodia sp.* and *S. bataticola* grow so rapidly under the conditions used for isolations it seems certain that they were not present in these particular seeds. The damage, therefore, could have resulted only from the action of the fungi isolated. In addition there is further evidence in table 2 that other fungi produce concealed damage. Each of two isolates of *Fusarium sp.* (isolates No. 5 and No. 6) were reisolated from some concealed-damage seeds after being used as inoculum. The high percentage of isolates of *Rhizopus sp.* and *Mucor sp.* from seeds from certain flasks indicates that these two fungi also may produce the condition, even though neither was reisolated from concealed-damage seeds when used as inoculum.

Of the fungi found to be associated with peanut fruits *Rhizopus sp.*, *Diplodia sp.*, and *Sclerotium bataticola* are capable of making the most rapid initial growth (Table 3). Thus, when curing peanuts are exposed to conditions favorable to fungus growth, the initial saprophytic activity of these three fungi should result in some concealed damage. Prolonged exposure to such conditions should bring other filamentous fungi and bacteria into action and result in further concealed damage followed by complete decay. Such a sequence of fungi is evident in table 3 where the other fungi appear to be "post-saprophytes" in that their period of major activity follows that of the three fungi mentioned.

Fungi capable of producing concealed damage have been demonstrated to be present in the fungus flora of peanut fruits when taken directly from the soil (Table 1). The fact that concealed damage develops in surface sterilized, noninoculated fruits when incubated (Table 2) shows that concealed damage may result from the action of fungi normally associated with the mature fruit. Consequently, any control methods must be aimed, not at keeping fungi away from curing peanuts, but rather at preventing the development of fungi already present. Work now in progress at this Station and in Alabama (8) indicates that the development of concealed damage may be reduced significantly by certain curing methods.

SUMMARY AND CONCLUSIONS

In a general study of concealed damage, the fungi associated with peanut fruits are being identified and tests are being made to determine which can produce concealed damage.

The fungi most frequently associated with peanut fruits are common soil-borne saprophytes such as *Rhizopus sp.*, *Aspergillus spp.*, *Sclerotium bataticola*, *Diplodia sp.*, and species of *Fusarium*. Species parasitic on peanuts are uncommon.

Uniformity between localities or fields in fungus flora of peanut fruits is not to be expected. Although certain prevalent fungi, such as *Diplodia sp.*, *Sclerotium bataticola*, and *Fusarium spp.*, appear consistently in this flora, antagonisms or other factors may result in the temporary elimination of one or more of these prevalent fungi.

Concealed damage results when a fungus invades the intercotyledonary space through the placenta, while most of the visible damage of a fungus nature results from the activity of fungi in the space between the shell and the seeds. A comparison of fungi isolated from visibly damaged seeds, from concealed-damage seeds, and from sound seeds indicates that much of the damage in each of the two types of damage is the result of the action of the same fungi, and that these fungi are normally associated with runner peanut fruits.

Antagonisms and competitions develop when peanut fruits with associated fungi are inoculated with a specific fungus. *Diplodia sp.* and *Sclerotium bataticola* were more vigorous in these artificially induced competitions and usually outgrew any other fungus used as inoculum. Similar antagonisms and competitions developing in soils and in curing stacks would account for the preponderance of *Diplodia sp.* and *S. bataticola* in isolations from concealed-damage seeds from fields.

Inoculation tests have shown that both *Diplodia sp.* and *Sclerotium bataticola* can produce concealed damage. There is evidence, however, that several other species of fungi associated with peanut seeds can produce this condition. Laboratory tests on these other fungi are made difficult by the presence of various fungi in the peanut fruit.

The initial growth of *Rhizopus sp.*, *Diplodia sp.*, and *Sclerotium bataticola* was more rapid than that of the other fungi associated with peanut seeds. When harvested peanuts are exposed to conditions favorable for the growth of fungi, concealed damage may result from the early saprophytic activity of these fungi.

Since fungi capable of producing concealed damage are associated with peanut fruits when the crop is taken from the soil, control methods for concealed damage are limited to preventing the growth of these fungi during the curing process.

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PHYTOPATHOLOGICAL NOTES

An Unusual Case of Clitocybe Root Rot in Ficus elastica Propagation Stock in a Florida Nursery.[1]—One of the most remarkable cases of mushroom root rot caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres. that the writer has seen in 20 years' observations on this disease in Florida was found in a stock planting of India-rubber fig (*Ficus elastica* Roxb.) and the variety *variegata* grown, for propagation of the tips for the northern trade, under a lath-shed at a large nursery near Apopka, Florida. The planting comprised nearly three-fourths of an acre of closely set stock plants. These had been planted about 2½ years and were about a year old when set out. To induce rooting, the stems back of the growing tips were packed with sphagnum moss before the tips were severed for potting. The plants were watered every day by means of overhead irrigation in order to keep the moss wet. About 6 weeks are required for the "mossed" branches to develop a good root system and after the crop is cut the treatment is repeated when new shoots develop. From 2 to 3 crops are thus secured a year.

Clitocybe root rot had been observed in this planting for about two years by Mr. John R. Springer, nursery inspector of the State Plant Board of Florida, to whom the writer is indebted for calling his attention to this case. The disease was said to be becoming progressively worse and it was estimated to have killed 20–25 per cent of the original stock planting, much of which had been reset from time to time, as well as some of the newly set stock. When this planting was examined with Mr. Springer on June 2, 1944, a large number of plants were found to be more or less completely girdled by the root-rot fungus and a considerable number of dead ones had been pulled up shortly before but not yet carried out. In some cases completely girdled plants continued to live because they had developed adventitious roots above the girdled bases. In many instances fresh clusters of the sporophores of *C. tabescens* had developed from the bases of attacked plants. The disease occurred scattered throughout a large part of the planting but was much worse on the higher portion, the planting being on a slope. Some idea of the loss occasioned by this disease may be secured from the fact that rooted tips were worth 20 cents apiece wholesale at the time and that an average of 5 rooted tips were secured a year from each plant.

The practice of sprinkling the plants daily to keep the moss layers wet unquestionably was highly conducive to infection and the extremely rapid development and spread of the disease. Owing to the sandy nature of the soil, however, there was no tendency to waterlogging.

By January, 1947, the disease had become so severe that growing these plants in this particular location was no longer profitable, and the small

[1] These observations were made while the writer was employed in Florida on the Emergency Plant Disease Prevention Project of the U. S. Department of Agriculture. The writer is at present Biologist, Industrial Test Laboratory, Philadelphia Naval Shipyard.

number remaining had been destroyed and other crops planted. The new crop of ferns does not appear to be injured by the fungus.

It is of interest that one plant enthusiast, who was troubled by frequent losses of cherished plants from Clitocybe root rot over a period of several years at his place at Lake Alfred, Florida, reported that after he discontinued watering his plants so frequently during dry weather when getting ready to move them to a new locality, he experienced a marked reduction in losses from this disease.

The efforts of the writer in making inoculations with *Clitocybe tabescens* in past years in Florida were severely handicapped by the prevalence of droughty soil conditions, which tended to make results very slow and uncertain. The exceptionally rapid spread and destructiveness of this fungus in the planting described above suggests that overhead irrigation or some other method of maintaining a high moisture content of the soil should prove of great value in conducting inoculation experiments.—ARTHUR S. RHOADS.

Breeding Wheat to Combine Resistance to Leaf Rust, Speckled Leaf Blotch, and Glume Blotch.[1]—The acreage of wheat in Arkansas has been reduced during the past decade or two primarily because of the prevalence of leaf rust (*Puccinia rubigo-vera* var. *tritici*), speckled leaf blotch (*Septoria tritici*), and glume blotch (*S. nodorum*).

In 1941 a start was made to breed wheat for disease resistance, not primarily for bread or pastry wheats but for winter pasturage and grain feed. Among the various wheats under study at the university farm, Fayetteville, was Fultz Sel. × Hungarian, C.I. 12017. It was noted that while this wheat had less leaf rust than any named variety, nearly all the plants had a few relatively large pustules (3–4 type) indicative of a susceptible reaction. However, a few plants with no such reaction appeared not only highly resistant to leaf rust but had fewer infections of *Septoria tritici* and *S. nodorum*. Of many varieties and selections of wheat under study, this was the first that seemingly had a combined resistance to the 3 diseases although its resistance to *S. tritici* was not of a very high type.

Acting on the theory that C.I. 12017 was not homozygous for resistance to these diseases and that the few plants noted as having no large leaf-rust pustules might represent segregates of a more resistant type, 6 single head selections were made from these in 1943.

Since the plants derived from these single head selections again lacked uniformity in disease resistance in 1944, single heads or single plants were selected once more in 1944, and in the succeeding generation in 1945. Thus three successive series of selections were made.

Compared with C.I. 12017 most of these selections, present in one or more rod rows in 1946, had a higher and more uniform type of resistance to leaf rust and to glume blotch, and somewhat more resistance to speckled leaf blotch. However, while some of these selections appeared uniformly resis-

[1] Research Paper No. 824, Journal Series, University of Arkansas.

tant, others were apparently still segregating. Of 37 such selections, 25 had a lower and more uniform rust reading than the original while 12 showed no increase in resistance. On April 25, when Purplestraw (Alabama Bluestem), which was used as a check every tenth row, and Sanford, which in former years had been resistant to leaf rust, gave readings of 35 to 50 per cent leaf rust, none of the selections of C.I. 12017 had more than a trace. On May 18–20, when Purplestraw and Sanford had fully 90 per cent of their upper blades occupied by leaf rust pustules and when their lower blades were dead, killed mostly by combined action of speckled-leaf-blotch and leaf-rust fungi, the highest reading obtained on the original C.I. 12017 and its selections was 20 per cent. Most of the selections had merely a trace of leaf rust, the pustules being few, very small, and of a type that led one to consider the selections intermediate between resistant and susceptible. However, the presence of a large number of chlorotic flecks on these selections without any pustules indicated a high type of leaf-rust resistance.

TABLE 1.—*Comparison between Selection 12017–24–2, C.I. 12017, and Sanford wheat in reaction to 8 race groups of the leaf-rust fungus*

Wheat selection or variety	Reaction[a] to race group:[b]							
	2	5	6	9	12	21	45	65
12017–24–2	X	X	R	R	R	R	R	R
C.I. 12017	S,X	S,X	R	R	R	X,R	S,R	R
Sanford	R,S	S	S	S	R	S	R	S

[a] Key: S = susceptible, R = resistant, X = intermediate or indeterminate, S,X; S,R; etc. = mixture of wheats with different reactions indicating varietal impurity or segregating condition.

[b] Chester combines a number of formerly recognized races into a race group; he includes in race group 2 the former races 2, 3, 15, 25, 34, 59, 62, 102, and 127; in group 5 he includes races 5 and 52; in group 6 he includes races 6, 28, 39, 105, and 126; in group 9 he includes races 9, 10, 13, 19, 20, 24, 27, 29, 31, 108, and 115; in group 12 he includes races 12, 32, 44, 58, 61, 76, 81, 84, 85, 88, and 90; in group 21 he includes races 21, 30, 35, 42, 54, 77, 89, 94, and 122; in group 45 he includes races 45, 57, and 67; and in group 65 he includes races 65, 80, and 101.

Since K. Starr Chester of the Oklahoma Agricultural and Mechanical College had very kindly offered to determine leaf-rust reaction to specific races, seed of the selection 12017–24–2 were sent to him along with seed of C.I. 12017 and of Sanford. The selection sent, while not having the highest type of leaf-rust resistance in 1946, leaf-rust infection having been read as 15 per cent on May 20, was the only one for which seed were available at the time of his testing. His results are presented in table 1.

While C.I. 12017 gave both a susceptible and an intermediate, or a susceptible and a resistant reaction to race groups 2, 5, 21, and 45, selection 12017–24–2 yielded a uniformly higher type of resistant reaction to all 4 race groups (Table 1). This is consistent with the field behavior of this selection and some of its sister selections.

Chester notes that 12017–24–2 is particularly interesting because of its high resistance to race group 21. He finds all 5 differential varieties which he uses in identifying races of leaf rust[2] are susceptible to this race group, indicating a wider degree of aggressiveness for this race group than for any of the others. Although he seemingly did not find this race group common in 1946[3] or previous years, it represents a threat to any susceptible variety where leaf rust is commonly destructive. The resistance of 12017–24–2, a soft red wheat, to race group 21 appears to be of some importance to future breeding work.

Aside from their resistance to leaf rust and partial resistance to the two Septorias, these selections were not injured by Hessian fly when planted early in the fall of 1945 and of 1946, a month prior to the fly-free date. The selections seem to possess more winter hardiness than Sanford, Sanett, Austin, and Purplestraw, and suffered much less winter injury during the severe freeze of December 29, 1946, to January 6, 1947. Their grain yields appear promising. Straw characters remain largely to be determined although they appear to be desirable. Most of these selections are beardless.

According to R. M. Caldwell (personal communication), C.I. 12017 represents a selection from the cross Fultz selection C.I. 11512 × Hungarian C.I. 4830–1, the cross having been made in 1929 by E. B. Mains and L. E. Compton at the Agricultural Experiment Station, Purdue University. F_2 seed were turned over to Caldwell in 1930 and he, with the help of Compton, made selections, the last one in the F_6 generation. Caldwell notes that the main genes for resistance to leaf rust were derived from Fultz C.I. 11512. This is a sister line to Wabash, both having been derived from a segregating line of Fultz C.I. 5308. He further notes that C.I. 12017 is more resistant than the Fultz parent and derives resistance to some races from the Hungarian parent (a synonym of Turkey according to Clark and Bayles[4]) from which it also inherited resistance to most races of bunt. However, C.I. 12017 is very susceptible to loose smut. Otherwise, according to Caldwell, it would have been released.

No adequate tests or significant field observations have been made of the selections here described relative to resistance to bunt or susceptibility to loose smut. Both of these diseases are usually of minor importance in Arkansas. Nevertheless, large increase in acreage of any wheat variety or of any other crop would probably give rise to pathological problems that are of no great importance at present.

Another breeding line that appears to offer hope of combined resistance to leaf rust and the two Septorias is represented in a cross (Red Rock × Hope) × C.I. 12017. The Red Rock × Hope parent represents a cross that was made by E. B. Mains in 1927 at the Agricultural Experiment Station,

[2] Chester, K. Starr. The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. 269 pp. Chronica Botanica, Waltham, Mass. 1946.

[3] Chester, K. Starr, and D. A. Preston. Another epiphytotic of fall wheat leaf rust in the southwest. U. S. Dept. Agr., Pl. Dis. Reptr., **31**: 15–17. 1947.

[4] Clark, J. Allen, and B. B. Bayles. Classification of wheat varieties grown in the United States in 1939. U. S. Dept. of Agr., Tech. Bul. 795. 1942.

Purdue University, and selected out by R. M. Caldwell and L. E. Compton of the same institution (personal communications from R. M. Caldwell and C. O. Johnston). It was further reselected by C. O. Johnston at Manhattan, Kansas, and placed in the uniform winter wheat rust nurseries in 1937. In 1941 and 1942, when leaf rust and speckled leaf blotch were severe at the university farm, Fayetteville, some plants of this cross were freer from these two diseases than most of the other plants. Fifteen single head selections were made from the resistant plants and their progeny grown from 1942 to 1946.

These selections while having fair resistance to *Septoria tritici* seemingly developed more and more leaf rust during these years. This also happened to most Hope crosses, the variety Austin being the exception, and it was probably due to the greater prevalence of races to which these Hope crosses were susceptible. In any case, selections from Red Rock × Hope were so badly rusted in 1946 that they are being discarded.

The cross (Red Rock × Hope) × C.I. 12017 was made by the writer in a greenhouse during the winter of 1942–43. The F_1 grown outdoors seemingly had a high degree of resistance to leaf rust and speckled leaf blotch. Not enough glume blotch was present to judge of resistance to this disease.

Single plant selections have been made in the F_2 and F_3, the current generation being the F_4, the selections again being made primarily for resistance to the 3 diseases. Since the lines are still segregating, there is no assurance that resistance to these diseases will be combined with other desirable characters. However, there appears to be little doubt that a higher type of resistance to *Septoria tritici* has been obtained in some of the selections coupled with a type of reaction to leaf rust that approaches immunity. In this type of reaction there are few or no chlorotic or necrotic flecks and a complete absence of pustules.

The cross was made with the hope of combining the higher type of resistance to speckled blotch to be found in the Red Rock × Hope parent with the leaf rust resistance, head characters, and better straw of C.I. 12017. It was also hoped that the stem rust resistance of Red Rock × Hope might be incorporated into this cross although this disease is usually not important in Arkansas. Up to the present there are insufficient data to indicate that this has been accomplished.—H. R. ROSEN, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

A Pythium Plate Method of Evaluating Fungicides.—A supplement to other methods for evaluating fungicides has been developed with *Pythium debaryanum* Hesse as the test organism.[1]

The fungus is grown on potato-dextrose agar (Difco prepared) for 5 days. A piece of the infested agar, one-half inch square, is placed in the center of a sterile Petri dish and seeded with approximately 50 sterilized wheat seeds. Five ml. sterile water were added to maintain sufficient mois-

[1] In the early stages L. Shanor contributed to developing this technique.

ture. The plates are held for 4 days at 24° C., by which time each seed is thoroughly infested.

Twenty ml. of the fungicide solution is then poured into a 10-cm. Petri dish and one infested wheat seed is placed in the center of the dish. Measurements of mycelial growth are made after 48 hours. A special glass-topped box containing a 60-watt electric bulb with a millimeter rule attached to the surface of the glass top was used for this purpose. Each plate is placed on the ruler and the distance from the seed to the perimeter of the mycelial growth is recorded. Plates containing 20 ml. distilled water and one infested seed are used as controls.

Ten compounds, including 8-quinolinol and 9 of its derivatives, were tested by this method. The same compounds were evaluated by the settling tower method of McCallan[2] and by the spore-dilution-slide method[3] using *Stemphylium sarcinaeforme* (Cav.) Wilts. as the test organism.

A comparison of the 10 compounds evaluated by the 3 procedures is shown in table 1. The *Stemphylium* spore-dilution method and the *Pythium*

TABLE 1.—*Rank of ten compounds evaluated by three laboratory assay procedures, employing two test organisms*

Compound	Method of assay				
	Stemphylium		*Pythium*		
	Tower	Dilution	Plate tests		
Copper 8-quinolinolate	1	1	1	1	1
Magnesium 8-quinolinolate	2	3	4	4	4
8-quinolinol	3	5	2	3	3
Zinc 8-quinolinolate	4	8	8	8	10
Manganese 8-quinolinolate	5	4	7	6	6
5,7, dibromo 8-quinolinol	6	6	5	5	5
5,7, dichloro 8-quinolinol	7	2	3	2	2
Copper, 5,7, dibromo 8-quinolinolate	8	7	6	7	7
Iron 8-quinolinolate	9	9	9	9	8
5,7, dinitro 8-quinolinol	10	10	10	10	9

plate method correspond favorably; differences between 2 test organisms of the degree shown in the table are not unusual. The 3 procedures show the same trend of toxicity. Since the *Pythium* plate method is simple and time saving, it should prove useful in screening compounds as fungicides.—CURTIS L. MASON and DWIGHT POWELL, Division of Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Illinois.

Influence of Fasting in the Transmission of the Beet-Mosaic Virus by the Green Peach Aphid.—Watson[1] published data which indicated that a period

[2] McCallan, S. E. A., and Frank Wilcoxon. An analysis of factors causing variation in spore germination tests. II. Methods of spraying. Contrib. Boyce Thompson Inst. 11: 309–324. 1940.

[3] The American Phytopathological Society, Committee on Standardization of Fungicidal Tests. The slide-germination method of evaluating protectant fungicides. Phytopath. 33: 627–632. 1943.

[1] Watson, M. A. Further studies on the relationship between *Hyoscyamus* virus 3 and the aphid *Myzus persicae* (Sulz.) with special reference to the effects of fasting. Proc. Roy. Soc. Lond., B. 125: 144–170. 1938.

of fasting prior to a short infection feeding resulted in an increase in vector efficiency in the transmission of a nonpersistent[2] virus. The virus used was Hyoscyamus virus 3 and the vector was the green peach aphid, *Myzus persicae* (Sulzer). Watson and Roberts[2] and Watson[3] have since published additional data regarding this phenomenon, and the results have been accepted generally in respect to validity. The evidence of Watson has been substantiated in England by another worker,[4] but to the knowledge of the author it has lacked confirmation among the virus workers in the United States.

Recently it has been possible to demonstrate the action of a period of fasting prior to an infection feeding, using the beet-mosaic virus, as the example of the nonpersistent virus, and the green peach aphid as the vector.

TABLE 1.—*Results*[a] *of trials to determine the influence of a fasting period prior to an infection feeding upon the ability of the green peach aphid to transmit the beet-mosaic virus*

Replication	Vector treatment	Infection feeding in minutes			Totals
		0.5	1.0	2.0	
A	Fasted	0/5	2/5	0/5	2/15
	Unfasted	0/5	0/5	0/5	0/15
B	Fasted	0/5	1/5	1/5	2/15
	Unfasted	0/5	0/5	0/5	0/15
C	Fasted	0/5	1/5	0/5	1/15
	Unfasted	0/5	0/5	0/5	0/15
D	Fasted	1/5	0/5	2/5	3/15
	Unfasted	0/5	0/5	1/5	1/15
E	Fasted	1/5	2/5	2/5	5/15
	Unfasted	2/5	0/5	0/5	2/15
Totals	Fasted	2/25[b]	6/25	5/25	13/75
	Unfasted	2/25	0/25	1/25	3/75

χ^2 6.98 for 1 degree of freedom.

[a] In the ratios listed, the numerator is the number of plants infected, while the denominator is the number of plants inoculated.

[b] In a second trial, using 6 replications, 5 plants per replication, the infection ratios obtained were: fasted, 15/30; unfasted, 2/30.

Apterae (wingless females) obtained from a noninfective colony of the green peach aphid established upon a sugar beet in the greenhouse were fasted in a vial for a minimum of 2 hours and a maximum of 4 hours. Following the completion of the starvation period, the infection feedings were begun. Single insects were fed upon a single virus source for infection feeding intervals of 0.5, 1.0, and 2.0 minutes. Five single aphids were fed

[2] Watson, M. A., and F. M. Roberts. A comparative study of the transmission of *Hyoscyamus* virus 3, potato virus Y and cucumber virus 1 by the vectors *Myzus persicae* (Sulz.), *M. circumflexus* (Buckton), and *Macrosiphum gei* (Koch). Proc. Roy. Soc. Lond., B, **127**: 543–576. 1939.

[3] Watson, M. A. The transmission of beet mosaic and beet yellow viruses by aphids; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proc. Roy. Soc. Lond., B, **133**: 200–219. 1946.

[4] Kassanis, B. Transmission of tobacco etch viruses by aphids. Ann. Appl. Biol. **28**: 238–243. 1941.

for each interval and then placed singly upon healthy beet seedlings for a 24-hour test feeding period. During the same day single apterae from the colony were selected and used in a like manner, with the exception that they were not fasted prior to the infection feedings.

The procedure was repeated in order to obtain 5 replications of each infection feeding interval with a comparison between previously fasted and unfasted individuals. The results are given in table 1.

The total number of infections obtained under the influence of fasting apparently was significant over the total obtained by unfasted vectors. However, the relationship was not demonstrated by the 0.5-minute interval. In order to test this interval further, 6 replications, using 5 plants in each replication, comparing fasted and unfasted apterae at the 0.5-minute interval, were made. In these trials, 2 out of the 30 plants became infected in the unfasted series, while 15 out of the 30 plants became infected in the fasted series.

The data indicate that the factor of fasting has a beneficial effect upon the ability of green peach aphid apterae to transmit the beet-mosaic virus, when used in conjunction with short infection feeding periods. Consequently, it is confirmatory evidence for the phenomenon initially described by Watson in connection with the Hyoscyamus virus 3 and subsequently for a number of other nonpersistent viruses.—E. S. SYLVESTER, Division of Entomology and Parasitology, University of California, Berkeley, California.

Berg's Rust-resistant Red Cedar Susceptible to Phomopsis juniperovora in Greenhouse Tests.—In 1940 Berg[1] reported a red cedar (*Juniperus virginiana* L.) clone highly resistant to cedar-apple rust caused by *Gymnosporangium juniperi-virginianae* Schw. The clone had been observed for 16 years in the field under conditions of natural infection and for an additional period of 4 years' exposure to rust under more closely controlled field conditions. The writer became interested in the Berg cedar with regard to its possible resistance to *Phomopsis juniperovora* Hahn[2] which causes the destructive blight of nursery cedars. In a letter to the writer Berg stated that from his field observations the rust-resistant cedar did not seem to be resistant to *Phomopsis*. In the spring of 1941 he generously provided the writer with 4 small rust-resistant red cedar grafts for controlled testing. The Berg clone was first cultivated in pots in an unheated greenhouse. Two of the plants died; the two remaining plants, which at first grew slowly, were transplanted finally to a floor bed in the same house, where they have grown vigorously, producing foliage of a very handsome bluish-green color.

The two rust-resistant cedars were inoculated in 1946 with an isolate of *Phomopsis juniperovora* taken originally from a diseased branchlet of a 25-year-old red cedar hedge at Manhattan, Kansas. This material had been collected in January, 1945, by C. M. Slagg and after storage in an ice box at

[1] Berg, A. A rust-resistant red cedar. Phytopath. **30**: 876–878. 1940.

[2] Hahn, G. G. Taxonomy, distribution, and pathology of *Phomopsis occulta* and *P. juniperovora*. Mycologia **35**: 112–129. 1943.

approximately 40° F., a number of monospore isolations of the parasite were made by the writer the following September. Isolate No. 1 of this series was used in October, 1945, to inoculate a susceptible red cedar. A reisolation of this isolate, made in August, 1946, was utilized again the following month for 8 slit-wound inoculations of the two trees of the Berg clone. The inoculations were made according to a method previously described,[3, p. 903] four to each tree, with accompanying checks. Infections resulted from all inoculations; the checks were not infected. Lateral branches, 3 to 4 mm. in diameter, inoculated close to the trunk, subsequently died back to the main axis. When inoculum was placed on the trunk, which was 7 mm. in diameter at the point of incision, the parasite girdled the trunk and slowly killed the terminal. However, the pathogen attacked tissue for only 2 cm. below the inoculation site. Within two months erumpent sporulating pycnidia were observed about the inoculation incisions. Reisolations in early December of *P. juniperovora* from diseased inner-bark tissue produced on Bacto malt extract agar[4, p. 455] yellow coloration and red crystals in the medium, two cultural characteristics that were first indicated[5] for the parasite 30 years ago and that proved to be specific. These constant characters have facilitated accurate identification of the species since its discovery.

Under the conditions of the test the Berg rust-resistant red cedar clone was not resistant to juniper blight. Moreover, the results indicate that the basis of resistance to the rust in this clone is not operative with respect to *Phomopsis juniperovora*.—GLENN GARDNER HAHN, Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, in cooperation with Osborn Botanical Laboratory, Yale University, New Haven, Conn.

[3] Hahn, G. G. *Phomopsis juniperovora* and closely related strains on conifers. Phytopath. **16**: 899–914. 1926.

[4] Levine, M., and H. W. Schoenlein. A compilation of culture media for the cultivation of microorganisms. 969 pp. Baltimore. 1930.

[5] Hahn, G. G., C. Hartley, and R. G. Pierce. A nursery blight of cedars. Jour. Agr. Res. [U.S.] **10**: 533–540. 1917.

ANNOUNCEMENT

The summer meeting of the Plant Pathologists of the Upper Mississippi Valley will be held August 6, 7, and 8, 1947, at the Ohio Experiment Station, Wooster, Ohio. The meeting will consist of exhibits, discussions, and a tour. A formal questionnaire has preceded this announcement. Special items to be discussed are: (1) Formal organization of a Division of The American Phytopathological Society, and (2) A plan for arranging regional projects.

H. C. YOUNG, *Chairman*
Upper Mississippi Valley Pathologists